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(71) Applicant *(for all designated States except US):* ISIS PHARMACEUTICALS, INC. [US/US]; 2280 Faraday Avenue, Carlsbad, CA 92008 (US).

(72) Inventors; and
(75) Inventors/Applicants *(for US only) :* BENNETT, Clarence, Frank [US/US]; 6553 Corte Cisco, Carlsbad, CA 92008 (US). MIRABELLI, Christopher, K. [US/US]; 1728 Shadow Mountain Drive, Encinitas, CA 92024 (US).

(74) Agents: CALDWELL, John, W. et al.; Woodcock Washburn Kurtz Mackiewicz & Norris, One Liberty Place, 46th Floor, Philadelphia, PA 19103 (US).

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(54) Title: OLIGONUCLEOTIDE MODULATION OF CELL ADHESION

(57) Abstract

Compositions and methods are provided for the treatment and diagnosis of diseases amenable to treatment through modulation of the synthesis or metabolism of intercellular adhesion molecules. In accordance with preferred embodiments, oligonucleotides and oligonucleotide analogs are provided which are specifically hybridizable with nucleic acids encoding intercellular adhesion molecule-1, vascular cell adhesion molecule-1, and endothelial leukocyte adhesion molecule-1. The oligonucleotide comprises nucleotide units sufficient in identity and number to effect said specific hybridization. In other preferred embodiments, the oligonucleotides are specifically hybridizable with a transcription initiation site, a translation initiation site, 5′-untranslated sequences, 3′-untranslated sequences, and intervening sequences. Methods of treating animals suffering from disease amenable to therapeutic intervention by modulating cell adhesion proteins with an oligonucleotide or oligonucleotide analog specifically hybridizable with RNA or DNA corresponding to one of the foregoing proteins are disclosed. Methods for treatment of diseases responding to modulation cell adhesion molecules are disclosed.

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OLIGONUCLEOTIDE MODULATION OF CELL ADHESION

FIELD OF THE INVENTION

This invention relates to diagnostics, research reagents, and therapies for disease states which respond to modulation of the synthesis or metabolism of cell adhesion molecules. In particular, this invention relates to antisense oligonucleotide interactions with certain messenger ribonucleic acids (mRNAs) or DNAs involved in the synthesis of proteins that regulate adhesion of white blood cells to other white blood cells and to other cell types. Antisense oligonucleotides designed to hybridize to the mRNA encoding intercellular adhesion molecule-1 (ICAM-1), endothelial leukocyte adhesion molecule-1 (ELAM-1), and vascular cell adhesion molecule-1 (VCAM-1) are provided. These oligonucleotides have been found to lead to the modulation of the activity of the RNA or DNA, and thus to the modulation of the synthesis and metabolism of specific cell adhesion molecules. Palliation and therapeutic effect result.

BACKGROUND OF THE INVENTION

Inflammation is a localized protective response elicited by tissues in response to injury, infection, or tissue destruction resulting in the destruction of the infectious or injurious agent and isolation of the injured tissue. A typical inflammatory response proceeds as follows: recognition of an antigen as foreign or recognition of tissue damage, synthesis and release of soluble inflammatory mediators, recruitment of inflammatory cells to the site of infection or tissue damage,

destruction and removal of the invading organism or damaged tissue, and deactivation of the system once the invading organism or damage has been resolved. In many human diseases with an inflammatory component, the normal, homeostatic mechanisms which attenuate the inflammatory responses are defective, resulting in damage and destruction of normal tissue.

Cell-cell interactions are involved in the activation of the immune response at each of the stages described above. One of the earliest detectable events in a normal inflammatory response is adhesion of leukocytes to the vascular endothelium, followed by migration of leukocytes out of the vasculature to the site of infection or injury. The adhesion of these leukocytes, or white blood cells, to vascular endothelium is an obligate step in the migration out of the vasculature. Harlan, J.M., *Blood* 65: 513-525 (1985). In general, the first inflammatory cells to appear at the site of inflammation are neutrophils followed by monocytes, and lymphocytes. Cell-cell interactions are also critical for propagation of both B-lymphocytes and T-lymphocytes resulting in enhanced humoral and cellular immune responses, respectively.

The adhesion of white blood cells to vascular endothelium and other cell types is mediated by interactions between specific proteins, termed "adhesion molecules," located on the plasma membrane of both white blood cells and vascular endothelium. The interaction between adhesion molecules is similar to classical receptor ligand interactions with the exception that the ligand is fixed to the surface of a cell instead of being soluble. The identification of patients with a genetic defect in leukocyte adhesion has enabled investigators to identify a family of proteins responsible for adherence of white blood cells. Leukocyte adhesion deficiency (LAD) is a rare autosomal trait characterized by recurrent bacterial infections and impaired pus formation and wound healing. The defect was shown to occur in the common B-subunit of

three heterodimeric glycoproteins, Mac-1, LFA-1, and p150,95, normally expressed on the outer cell membrane of white blood cells. Anderson and Springer, *Ann. Rev. Med.* 38:175-194 (1987). Patients suffering from LAD exhibit a defect in a wide spectrum of adherence-dependent functions of granulocytes, monocytes, and lymphocytes. Two ligands for LFA-1 have been identified, intercellular adhesion molecules 1 and 2 (ICAM-1 and ICAM-2). Both Mac-1 and p150,95 bind complement fragment C3bi and perhaps other unidentified ligands.

Other adhesion molecules have been identified which are involved in the adherence of white blood cells to vascular endothelium and subsequent migration out of the vasculature. These include endothelial leukocyte adhesion molecule-1 (ELAM-1), vascular cell adhesion molecule-1 (VCAM-1) and granule membrane protein-140 (GMP-140) and their respective receptors. The adherence of white blood cells to vascular endothelium appears to be mediated in part if not *in toto* by the five cell adhesion molecules ICAM-1, ICAM-2, ELAM-1, VCAM-1 and GMP-140. Dustin and Springer, *J. Cell Biol.*, 107:321-331 (1987). Expression on the cell surface of ICAM-1, ELAM-1, VCAM-1 and GMP-140 adhesion molecules is induced by inflammatory stimuli. In contrast, expression of ICAM-2 appears to be constitutive and not sensitive to induction by cytokines. In general, GMP-140 is induced by autocoids such as histamine, leukotriene B_4, platelet activating factor, and thrombin. Maximal expression on endothelial cells is observed 30 minutes to 1 hour after stimulation and returns to baseline within 2 to 3 hours. The expression of ELAM-1 and VCAM-1 on endothelial cells is induced by cytokines such as interleukin-1ß and tumor necrosis factor, but not gamma-interferon. Maximal expression of ELAM-1 on the surface of endothelial cells occurs 4 to 6 hours after stimulation and returns to baseline by 16 hours. ELAM-1 expression is dependent on new mRNA and protein synthesis. Elevated VCAM-1 expression is detectable 2 hours following treatment

with tumor necrosis factor, is maximal 8 hours following stimulation, and remains elevated for at least 48 hours following stimulation. Rice and Bevilacqua, *Science*, 246:1303-1306 (1989). ICAM-1 expression on endothelial cells is induced by cytokines interleukin-1 tumor necrosis factor and gamma-interferon. Maximal expression of ICAM-1 follows that of ELAM-1 occurring 8 to 10 hours after stimulation and remains elevated for at least 48 hours.

GMP-140 and ELAM-1 are primarily involved in the adhesion of neutrophils to vascular endothelial cells. VCAM-1 primarily binds T and B lymphocytes. In addition, VCAM-1 may play a role in the metastasis of melanoma, and possibly other cancers. ICAM-1 plays a role in adhesion of neutrophils to vascular endothelium, as well as adhesion of monocytes and lymphocytes to vascular endothelium, tissue fibroblasts and epidermal keratinocytes. ICAM-1 also plays a role in T-cell recognition of antigen presenting cell, lysis of target cells by natural killer cells, lymphocyte activation and proliferation, and maturation of T cells in the thymus. In addition, recent data have demonstrated that ICAM-1 is the cellular receptor for the major serotype of rhinovirus, which account for greater than 50% of common colds. Staunton et al., *Cell*, 56: 849-853 (1989); Greve et al., *Cell*, 56: 839-847 (1989).

Expression of ICAM-1 has been associated with a variety of inflammatory skin disorders such as allergic contact dermatitis, fixed drug eruption, lichen planus, and psoriasis; Ho et al., *J. Am. Acad. Dermatol.*, 22: 64-68 (1990); Griffiths and Nickoloff, *Am. J. Pathology*, 135: 1045-1053 (1989); Lisby et al., *Br. J. Dermatol.*, 120:479-484 (1989); Shiohara et al., *Arch. Dermatol.*, 125: 1371-1376 (1989). In addition, ICAM-1 expression has been detected in the synovium of patients with rheumatoid arthritis; Hale et al., *Arth. Rheum.*, 32: 22-30 (1989), pancreatic B-cells in diabetes; Campbell et al., *Proc. Natl. Acad. Sci. U.S.A.*, 86:4282-4286 (1989); thyroid follicular cells in patients with Graves' disease; Weetman

et al., *J. Endocrinol.*, 122: 185-191 (1989); and with renal and liver allograft rejection; Faull and Russ, *Transplantation*, 48: 226-230 (1989); Adams et al., *Lancet*, 1122-1125 (1989).

It is has been hoped that inhibitors of ICAM-1, VCAM-1 and ELAM-1 expression would provide a novel therapeutic class of anti-inflammatory agents with activity towards a variety of inflammatory diseases or diseases with an inflammatory component such as asthma, rheumatoid arthritis, allograft rejections, various dermatological conditions, and psoriasis. In addition, inhibitors of ICAM-1, VCAM-1, and ELAM-1 may also be effective in the treatment of colds due to rhinovirus infection, AIDS, and some cancers and their metastasis. To date, there are no known therapeutic agents which effectively prevent the expression of the cellular adhesion molecules ELAM-1, VCAM-1 and ICAM-1. The use of neutralizing monoclonal antibodies against ICAM-1 in animal models provide evidence that such inhibitors if identified would have therapeutic benefit for asthma; Wegner et al., *Science*, 247:456-459 (1990) and renal allografts; Cosimi et al., *J. Immunol.*, 144:4604-4612 (1990). The use of a soluble form of ICAM-1 molecule was also effective in preventing rhinovirus infection of cells in culture. Marlin et al., *Nature*, 344:70-72 (1990).

Current agents which affect intercellular adhesion molecules include synthetic peptides, monoclonal antibodies, and soluble forms of the adhesion molecules. To date, synthetic peptides which block the interactions with ICAM-1, VCAM-1 or ELAM-1 have not been identified. Monoclonal antibodies may prove to be useful for the treatment of acute inflammatory response due to expression of ICAM-1, VCAM-1 and ELAM-1. However, with chronic treatment, the host animal develops antibodies against the monoclonal antibodies thereby limiting their usefulness. In addition, monoclonal antibodies are large proteins which may have difficulty in gaining access to the inflammatory

site. Soluble forms of the cell adhesion molecules suffer from many of the same limitations as monoclonal antibodies in addition to the expense of their production. Thus, there is a long felt need for molecules which effectively inhibit intercellular adhesion molecules. Antisense oligonucleotides avoid many of the pitfalls of current agents used to block the effects of ICAM-1, VCAM-1 and ELAM-1.

PCT/US90/02357 (Hession et al.) discloses DNA sequences encoding Endothelial Adhesion Molecules (ELAMs), including ELAM-1 and VCAM-1 and VCAM-1b. A number of uses for these DNA sequences are provided, including (1) production of monoclonal antibody preparations that are reactive for these molecules which may be used as therapeutic agents to inhibit leukocyte binding to endothelial cells; (2) production of ELAM peptides to bind to the ELAM ligand on leukocytes which, in turn, may bind to ELAM on endothelial cells, inhibiting leukocyte binding to endothelial cells; (3) use of molecules binding to ELAMS (such as anti-ELAM antibodies, or markers such as the ligand or fragments of it) to detect inflammation; (4) use of ELAM and ELAM ligand DNA sequences to produce nucleic acid molecules that intervene in ELAM or ELAM ligand expression at the translational level using antisense nucleic acid and ribozymes to block translation of a specific mRNA either by masking mRNA with antisense nucleic acid or cleaving it with a ribozyme. It is disclosed that coding regions are the targets of choice. For VCAM-1, AUG is believed to be most likely; a 15-mer hybridizing to the AUG site is specifically disclosed in Example 17.

OBJECTS OF THE INVENTION

It is a principle object of the invention to provide therapies for diseases with an immunological component, allografts, cancers and metastasis, colds, and AIDS through perturbation in the synthesis and expression of inflammatory cell adhesion molecules.

It is a further object of the invention to provide antisense oligonucleotides or oligonucleotide analogs which are capable of inhibiting the function of nucleic acids encoding intercellular adhesion proteins.

Yet another object is to provide means for diagnosis of dysfunctions of intercellular adhesion.

These and other objects of this invention will become apparent from a review of the instant specification.

BRIEF DESCRIPTION OF THE DRAWINGS

FIGURE 1 is the mRNA sequence of human intercellular adhesion molecule-1 (ICAM-1).

FIGURE 2 is the mRNA sequence of human endothelial leukocyte adhesion molecule-1 (ELAM-1).

FIGURE 3 is the mRNA sequence of human vascular cell adhesion molecule-1 (VCAM-1)

FIGURE 4 is a graphical representation of the induction of ICAM-1 expression on the cell surface of various human cell lines by interleukin-1 and tumor necrosis factor.

FIGURE 5 is a graphical representation of the effects of selected antisense oligonucleotides on ICAM-1 expression on human umbilical vein endothelial cells.

FIGURE 6A and 6B are a graphical representation of the effects of an antisense oligonucleotide on the expression of ICAM-1 in human umbilical vein endothelial cells stimulated with tumor necrosis factor and interleukin-1.

FIGURE 7 is a graphical representation of the effect of antisense oligonucleotides on ICAM-1 mediated adhesion of DMSO differentiated HL-60 cells to control and tumor necrosis factor treated human umbilical vein endothelial cells.

FIGURE 8 is a graphical representation of the effects of selected antisense oligonucleotides on ICAM-1 expression in A549 human lung carcinoma cells.

FIGURE 9 is a graphical representation of the effects of selected antisense oligonucleotides on ICAM-1 expression in primary human keratinocytes.

FIGURE 10 is a graphical representation of the relationship between oligonucleotide chain length, Tm and effect on inhibition of ICAM-1 expression.

FIGURE 11 is a graphical representation of the effect of selected antisense oligonucleotides on ICAM-1 mediated adhesion of DMSO differentiated HL-60 cells to control and tumor necrosis factor treated human umbilical vein endothelial cells.

FIGURE 12 is a graphical representation of the effects of selected antisense oligonucleotides on ELAM-1 expression on tumor necrosis factor-treated human umbilical vein endothelial cells.

SUMMARY OF THE INVENTION

In accordance with the present invention, oligonucleotides and oligonucleotide analogs are provided which specifically hybridize with nucleic acids encoding intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1) and endothelial leukocyte adhesion molecule-1 (ELAM-1). The oligonucleotides and oligonucleotide analogs are designed to bind either directly to mRNA or to a selected DNA portion forming a triple stranded structure, thereby modulating the amount of mRNA made from the gene.

The former relationship is commonly denoted as "antisense." The oligonucleotides and oligonucleotide analogs are able to inhibit the function of RNA or DNA, either its translation into protein, its translocation into the cytoplasm, or any other activity necessary to its overall biological function. The failure of the RNA or DNA to perform all or part of its function results in failure of a portion of the genome controlling cell adhesion molecules to be properly expressed, thus modulating said metabolism.

It is preferred to target specific genes for antisense attack. It has been discovered that the genes coding for ICAM-1, VCAM-1 and ELAM-1 are particularly useful for this approach. Inhibition of ICAM-1, VCAM-1 and ELAM-1 expression is expected to be useful for the treatment of inflammatory diseases, diseases with an inflammatory component, allografts, cancers and their metastasis, and viral infections.

Methods of modulating cell adhesion comprising contacting the animal with an oligonucleotide or oligonucleotide analog hybridizable with nucleic acids encoding a protein capable of modulating cell adhesion are provided. Oligonucleotides or oligonucleotide analogs hybridizable with an RNA or DNA encoding ICAM-1, VCAM-1 and ELAM-1 are preferred. Methods for diagnosis are also a part of this invention.

DETAILED DESCRIPTION OF PREFERRED EMBODIMENTS

Antisense oligonucleotides hold great promise as therapeutic agents for the treatment of many human diseases. Conceptually, it is much easier to design compounds which interact with a primary structure of a molecule such as an RNA molecule by base pairing than it is to design a molecule to interact with the active site of an enzyme or ligand binding site of a receptor. Oligonucleotides specifically bind to the complementary sequence of either pre-mRNA or mature mRNA, as defined by Watson-Crick base pairing, inhibiting the flow of genetic information from DNA to protein. The properties of antisense oligonucleotides which make them specific for their target sequence also make them extraordinarily versatile. Because antisense oligonucleotides are long chains of four monomeric units they may be readily synthesized for any target RNA sequence. Numerous recent studies have documented the utility of antisense oligonucleotides as biochemical tools for studying target proteins. Rothenberg et al., *J. Natl. Cancer Inst.*, 81:1539-1544 (1989); Zon, G. *Pharmaceutical Res.*, 5:539-

549). Because of recent advances in oligonucleotide chemistry, synthesis of nuclease resistant oligonucleotides, and oligonucleotide analogs which exhibit enhanced cell uptake, it is now possible to consider the use of antisense oligonucleotides as a novel form of therapeutics.

Antisense oligonucleotides offer an ideal solution to the problems encountered in prior art approaches. They can be designed to selectively inhibit a given isoenzyme, they inhibit the production of the enzyme, and they avoid non-specific mechanisms such as free radical scavenging or binding to multiple receptors. A complete understanding of enzyme mechanisms or receptor-ligand interactions is not needed to design specific inhibitors.

DESCRIPTION OF TARGETS

The acute infiltration of neutrophils into the site of inflammation appears to be due to increased expression of GMP-140, ELAM-1 and ICAM-1 on the surface of endothelial cells. The appearance of lymphocytes and monocytes during the later stages of an inflammatory reaction appear to be mediated by VCAM-1 and ICAM-1. ELAM-1 and GMP-140 are transiently expressed on vascular endothelial cells, while VCAM-1 and ICAM-1 are chronically expressed.

Human ICAM-1 is encoded by a 3.3-kb mRNA resulting in the synthesis of a 55,219 dalton protein (Figure 1). ICAM-1 is heavily glycosylated through N-linked glycosylation sites. The mature protein has an apparent molecular mass of 90 kDa as determined by SDS-polyacrylamide gel electrophoresis. Staunton et al., *Cell*, 52:925-933. ICAM-1 is a member of the immunoglobulin supergene family, containing 5 immunoglobulin-like domains at the amino terminus, followed by a transmembrane domain and a cytoplasmic domain. The primary binding site for LFA-1 and rhinovirus are found in the first immunoglobulin-like domain. However, the binding sites appear to be distinct. Staunton et al., *Cell*, 61:243-354 (1990).

Recent electron micrographic studies demonstrate that ICAM-1 is a bent rod 18.7 nm in length and 2 to 3 nm in diameter. Staunton et al., *Cell*, 61:243-254 (1990).

ICAM-1 exhibits a broad tissue and cell distribution, and may be found on white blood cells, endothelial cells, fibroblast, keratinocytes and other epithelial cells. The expression of ICAM-1 can be regulated on vascular endothelial cells, fibroblasts, keratinocytes, astrocytes and several cell lines by treatment with bacterial lipopolysaccharide and cytokines such as interleukin-1, tumor necrosis factor, gamma-interferon, and lymphotoxin. *See, e.g.*, Frohman, et al., *J. Neuroimmunol.*, 23:117-124 (1989). The molecular mechanism for increased expression of ICAM-1 following cytokine treatment has not been determined.

ELAM-1 is a 115-kDa membrane glycoprotein (Figure 2) which is a member of the selectrin family of membrane glycoproteins. Bevilacqua et al., *Science*, 243:1160-1165 (1989). The amino terminal region of ELAM-1 contains sequences with homologies to members of lectin-like proteins, followed by a domain similar to epidermal growth factor, followed by six tandem 60-amino acid repeats similar to those found in complement receptors 1 and 2. These features are also shared by GMP-140 and MEL-14 antigen, a lymphocyte homing antigen. ELAM-1 is encoded for by a 3.9-kb mRNA. The 3'-untranslated region of ELAM-1 mRNA contains several sequence motifs ATTTA which are responsible for the rapid turnover of cellular mRNA consistent with the transient nature of ELAM-1 expression.

ELAM-1 exhibits a limited cellular distribution in that it has only been identified on vascular endcthelial cells. Like ICAM-1, ELAM-1 is inducible by a number of cytokines including tumor necrosis factor, interleukin-1 and lymphotoxin and bacterial lipopolysaccharide. In contrast to ICAM-1, ELAM-1 is not induced by gamma-interferon. Bevilacqua et al., *Proc. Natl. Acad. Sci. USA*, 84:9238-9242 (1987); Wellicome et al., *J. Immunol.*,

144:2558-2565 (1990). The kinetics of ELAM-1 mRNA induction and disappearance in human umbilical vein endothelial cells precedes the appearance and disappearance of ELAM-1 on the cell surface. As with ICAM-1 the molecular mechanism for ELAM-1 induction is not known.

VCAM-1 is a 110-kDa membrane glycoprotein encoded by a 3.2-kb mRNA (Figure 3). VCAM-1 appears to be encoded by a single-copy gene. Osborn et al., *Cell*, 59:1203-1211 (1989). Like ICAM-1, VCAM-1 is a member of the immunoglobulin supergene family, containing six immunoglobulin-like domains of the H type. The receptor for VCAM-1 is proposed to be CD29 as demonstrated by the ability of monoclonal antibodies to CD29 to block adherence of Ramos cells to VCAM-1. VCAM-1 is expressed primarily on vascular endothelial cells. Like ICAM-1 and ELAM-1, expression of VCAM-1 on vascular endothelium is regulated by treatment with cytokines. Rice and Bevilacqua, *Science*, 246: 1303-1306 (1989); Rice et al., *J. Exp. Med.*, 171:1369-1374 (1990). Increased expression appears to be due to induction of the mRNA.

For therapeutics, an animal suspected of having a disease which can be treated by decreasing the expression of ICAM-1, VCAM-1 and ELAM-1 is treated by administering oligonucleotides or oligonucleotide analogs in accordance with this invention. Persons of ordinary skill can easily determine optimum dosages, dosing methodologies and repetition rates. Such treatment is generally continued until either a cure is effected or a diminution in the disease state is achieved. Long term treatment is likely for some diseases.

The present invention employs oligonucleotides and oligonucleotide analogs for use in antisense inhibition of the function of RNA and DNA corresponding to proteins capable of modulating inflammatory cell adhesion. In the context of this invention, the term "oligonucleotide" refers to a polynucleotide formed from naturally occurring bases and furanosyl groups joined by native phosphodiester

bonds. This term effectively refers to naturally occurring species or synthetic species formed from naturally occurring subunits or their close homologs.

"Oligonucleotide analog," as that term is used in connection with this invention, refers to moieties which function similarly to oligonucleotides but which have non-naturally occurring portions. Thus, oligonucleotide analogs may have altered sugar moieties or inter-sugar linkages. Exemplary among these are the phosphorothioate and other sulfur-containing species which are known for use in the art. In accordance with some preferred embodiments, at least some of the phosphodiester bonds of the oligonucleotide have been substituted with a structure which functions to enhance the ability of the compositions to penetrate into the region of cells where the RNA or DNA whose activity to be modulated is located. It is preferred that such substitutions comprise phosphorothioate bonds, methyl phosphonate bonds, or short chain alkyl or cycloalkyl structures. In accordance with other preferred embodiments, the phosphodiester bonds are substituted with other structures which are, at once, substantially non-ionic and non-chiral, or with structures which are chiral and enantiomerically specific. Persons of ordinary skill in the art will be able to select other linkages for use in practice of the invention.

Oligonucleotide analogs may also include species which include at least some modified base forms. Thus, purines and pyrimidines other than those normally found in nature may be so employed. Similarly, modifications on the furanosyl portions of the nucleotide subunits may also occur as long as the essential tenets of this invention are adhered to. Examples of such modifications are 2'-O-alkyl- and 2'-halogen-substituted nucleotides.

Such analogs are best described as being functionally interchangeable with natural oligonucleotides (or oligonucleotides synthesized along natural lines), but which have one or more differences from natural structure.

All such analogs are comprehended by this invention so long as they function effectively to hybridize with RNA and DNA deriving from a gene corresponding to one of the proteins capable of modulating intercellular adhesion. The oligonucleotides and oligonucleotide analogs in accordance with this invention preferably comprise from about 3 to about 50 subunits. It is more preferred that such oligonucleotides and oligonucleotide analogs comprise from about 8 to 25 subunits, and still more preferred to have from about 12 to 22 subunits. As will be appreciated, a subunit is a base-sugar combination suitably bound to adjacent subunits through phosphodiester or other bonds.

The oligonucleotides and oligonucleotide analogs used in accordance with this invention may be conveniently and routinely made through the well-known technique of solid phase synthesis. Equipment for such synthesis is sold by several vendors including Applied Biosystems. Any other means for such synthesis may also be employed, however the actual synthesis of the oligonucleotides is well within the talents of the routineer. It is also well known to use similar techniques to prepare other oligonucleotide analogs such as the phosphorothioates and alkylated derivatives.

In accordance with this invention, persons of ordinary skill in the art will understand that messenger RNA identified by the open reading frames (ORFs) of the DNA from which they are transcribed includes not only the information from the ORFs of the DNA, but also associated ribonucleotides which form regions known to such persons as the 5'-untranslated region, the 3'- untranslated region, and intervening sequence ribonucleotides. Thus, oligonucleotides and oligonucleotide analogs may be formulated in accordance with this invention which are targeted wholly or in part to these associated ribonucleotides as well as to the informational ribonucleotides. In preferred embodiments, the oligonucleotide or oligonucleotide analog is specifically

hybridizable with a transcription initiation site, a translation initiation site, an intervening sequence and sequences in the 3'-untranslated region.

In accordance with this invention, the oligonucleotide is specifically hybridizable with portions of nucleic acids encoding a protein involved in the adhesion of white blood cells either to other white blood cells or other cell types. In preferred embodiments, said proteins are intercellular adhesion molecule-1, vascular cell adhesion molecule-1 and endothelial leukocyte adhesion molecule-1. Oligonucleotides or analogs comprising the corresponding sequence, or part thereof, are useful in the invention. For example, Figure 1 is a human intercellular adhesion molecule-1 mRNA sequence. A preferred sequence segment which may be useful in whole or in part is:

5' 3'

TGGGAGCCATAGCGAGGC
GAGGAGCTCAGCGTCGACTG
GACACTCAATAAATAGCTGGT
GAGGCTGAGGTGGGAGGA
CGATGGGCAGTGGGAAAG
GGGCGCGTGATCCTTATAGC
CATAGCGAGGCTGAGGTTGC
CGGGGGCTGCTGGGAGCCAT
TCAGGGAGGCGTGGCTTGTG
CCTGTCCCGGGATAGGTTCA
TTGAGAAAGCTTTATTAACT
CCCCCACCACTTCCCCTCTC.

Figure 2 is a human endothelial leukocyte adhesion molecule-1 mRNA sequence. A preferred sequence segment which may be useful in whole or in part is:

5' 3'

CAATCATGACTTCAAGAGTTCT
TCACTGCTGCCTCTGTCTCAGG
TGATTCTTTTGAACTTAAAAGGA
TTAAAGGATGTAAGAAGGCT
CATAAGCACATTTATTGTC

TTTTGGGAAGCAGTTGTTCA
AACTGTGAAGCAATCATGACT
CCTTGAGTGGTGCATTCAACCT
AATGCTTGCTCACACAGGCATT.

Figure 3 is a human vascular cell adhesion molecule-1 mRNA sequence. A preferred sequence segment which may be useful in whole or in part is:

5' 3'
CCAGGCATTTTAAGTTGCTGT
CCTGAAGCCAGTGAGGCCCG
GATGAGAAAATAGTGGAACCA
CTGAGCAAGATATCTAGAT
CTACACTTTTGATTTCTGT
TTGAACATATCAAGCATTAGCT
TTTACATATGTACAAATTATGT
AATTATCACTTTACTATACAAA
AGGGCTGACCAAGACGGTTGT.

While the illustrated sequences are believed to be accurate, the present invention is directed to the correct sequences should errors be found. Oligonucleotides or analogs useful in the invention comprise one of these sequences, or part thereof. Thus, it is preferred to employ any of these oligonucleotides (or their analogs) as set forth above or any of the similar oligonucleotides or oligonucleotide analogs which persons of ordinary skill in the art can prepare from knowledge of the preferred antisense targets for the modulation of the synthesis of inflammatory cell adhesion molecules.

Several preferred embodiments of this invention are exemplified in accordance with the following nonlimiting examples. The target mRNA species for modulation relates to intercellular adhesion molecule-1, endothelial leukocyte adhesion molecule-1, and vascular cell adhesion molecule-1. Persons of ordinary skill in the art will appreciate that the present invention is not so limited, however, and that it is generally applicable. The inhibition or modulation of production of the ICAM-1 and/or

ELAM-1 and/or VCAM-1 are expected to have significant therapeutic benefits in the treatment of disease. In order to assess the effectiveness of the compositions, an assay or series of assays is required.

EXAMPLES

EXAMPLE 1

Expression of ICAM-1, VCAM-1 and ELAM-1 on the surface of cells can be quantitated using specific monoclonal antibodies in an ELISA. Cells are grown to confluence in 96 well microtiter plates. The cells are stimulated with either interleukin-1 or tumor necrosis factor for 4 to 8 hours to quantitate ELAM-1 and 8 to 24 hours to quantitate ICAM-1 and VCAM-1. Following the appropriate incubation time with the cytokine, the cells are gently washed three times with a buffered isotonic solution containing calcium and magnesium such as Dulbecco's phosphate buffered saline (D-PBS). The cells are then directly fixed on the microtiter plate with 1 to 2% paraformaldehyde diluted in D-PBS for 20 minutes at 25° C. The cells are washed again with D-PBS three times. Nonspecific binding sites on the microtiter plate are blocked with 2% bovine serum albumin in D-PBS for 1 hour at 37° C. Cells are incubated with the appropriate monoclonal antibody diluted in blocking solution for 1 hour at 37°C. Unbound antibody is removed by washing the cells three times with D-PBS. Antibody bound to the cells is detected by incubation with a 1:1000 dilution of biotinylated goat anti-mouse IgG (Bethesda Research Laboratories, Gaithersberg, MD) in blocking solution for 1 hour at 37°C. Cells are washed three times with D-PBS and then incubated with a 1:1000 dilution of streptavidin conjugated to ß-galactosidase (Bethesda Research Laboratories) for 1 hour at 37°C. The cells are washed three times with D-PBS for 5 minutes each. The amount of ß-galactosidase bound to the specific monoclonal antibody is determined by developing the plate in a solution of 3.3 mM chlorophenolred-ß-D-galactopyranoside, 50 mM sodium phosphate, 1.5 mM $MgCl_2$;

pH=7.2 for 2 to 15 minutes at 37°C. The concentration of the product is determined by measuring the absorbance at 575 nm in an ELISA microtiter plate reader.

An example of the induction of ICAM-1 observed following stimulation with either interleukin-1ß or tumour necrosis factor α in several human cell lines is shown in Figure 4. Cells were stimulated with increasing concentrations of interleukin-1 or tumour necrosis factor for 15 hours and processed as described above. ICAM-1 expression was determined by incubation with a 1:1000 dilution of the monoclonal antibody 84H10 (Amac Inc., Westbrook, ME). The cell lines used were passage 4 human umbilical vein endothelial cells (HUVEC), a human epidermal carcinoma cell line (A431), a human melanoma cell line (SK-MEL-2) and a human lung carcinoma cell line (A549). ICAM-1 was induced on all the cell lines, however, tumor necrosis factor was more effective than interleukin-1 in induction of ICAM-1 expression on the cell surface (Figure 4).

Screening antisense oligonucleotides for inhibition of ICAM-1, VCAM-1 or ELAM-1 expression is performed as described above with the exception of pretreatment of cells with the oligonucleotides prior to challenge with the cytokines. An example of antisense oligonucleotide inhibition of ICAM-1 expression is shown in Figure 5. Human umbilical vein endothelial cells (HUVEC) were treated with increasing concentration of oligonucleotide diluted in Opti MEM (GIBCO, Grand Island, NY) containing 16 μM N-[1-(2,3-dioleyloxy) propyl]-N,N,N-trimethylammonium chloride (DOTMA) for 4 hours at 37°C to enhance uptake of the oligonucleotides. The medium was removed and replaced with endothelial growth medium (EGM-UV; Clonetics, San Diego, CA) containing the indicated concentration of oligonucleotide for an additional 4 hours. Interleukin- 1β was added to the cells at a concentration of 5 units/ml and incubated for 14 hours at 37°C. The cells were quantitated for ICAM-1 expression using a 1:1000

dilution of the monoclonal antibody 84H10 as described above. The oligonucleotides used were:

COMPOUND 1 - (ISIS 1558) a phosphodiester oligonucleotide designed to hybridize with position 64-80 of the mRNA covering the AUG initiation of translation codon having the sequence

5'-TGGGAGCCATAGCGAGGC-3'.

COMPOUND 2 - (ISIS 1570) a phosphorothioate containing oligonucleotide corresponding to the same sequence as COMPOUND 1.

COMPOUND 3 - a phosphorothioate oligonucleotide complementary to COMPOUND 1 and COMPOUND 2 exhibiting the sequence

5'-GCCTCGCTATGGCTCCCA-3'.

COMPOUND 4 - (ISIS 1572) a phosphorothioate containing oligonucleotide designed to hybridize to positions 2190-2210 of the mRNA in the 3'untranslated region containing the sequence

5'-GACACTCAATAAATAGCTGGT-3'.

COMPOUND 5 - (ISIS 1821) a phosphorothioate containing oligonucleotide design to hybridize to human 5-lipoxygenase mRNA used as a control containing the sequence

5'-CATGGCGCGGGCCGCGGG-3'.

The phosphodiester oligonucleotide targeting the AUG initiation of translation region of the human ICAM-1 mRNA (COMPOUND 1) failed to inhibit expression of ICAM-1, however, the corresponding phosphorothioate containing oligonucleotide (COMPOUND 2) inhibited ICAM-1 expression by 70% at a concentration of 0.1 μM and 90% at 1 μM concentration (Figure 4). The increased potency of the phosphorothioate oligonucleotide over the phosphodiester is probably due to increased stability. The sense strand to COMPOUND 2, COMPOUND 3, modestly inhibited ICAM-1 expression at 10 μM. If COMPOUND 2 was prehybridized to COMPOUND 3 prior to addition to the cells, the effects of COMPOUND 2 on ICAM-1 expression were attenuated suggesting that the activity of COMPOUND 2 was due to antisense

oligonucleotide effect, requiring hybridization to the mRNA. The antisense oligonucleotide directed against 3' untranslated sequences (COMPOUND 4) inhibited ICAM-1 expression 62% at a concentration of 1 μM (Figure 5). The control oligonucleotide, targeting human 5-lipoxygenase (COMPOUND 5) reduced ICAM-1 expression by 20%. These data demonstrate that oligonucleotides are capable of inhibiting ICAM-1 expression on human umbilical vein endothelial cells and suggest that the inhibition of ICAM-1 expression is due to an antisense activity.

The antisense oligonucleotide COMPOUND 2 at a concentration of 1 μM inhibits expression of ICAM-1 on human umbilical vein endothelial cells stimulated with increasing concentrations of tumor necrosis factor and interleukin-1 (Figure 6). These data demonstrate that the effects of COMPOUND 2 are not specific for interleukin-1 stimulation of cells.

Analogous assays can also be used to demonstrate inhibition of ELAM-1 and VCAM-1 expression by antisense oligonucleotides.

EXAMPLE 2

A second cellular assay which can be used to demonstrate the effects of antisense oligonucleotides on ICAM-1, VCAM-1 or ELAM-1 expression is a cell adherence assay. Target cells are grown as a monolayer in a multiwell plate, treated with oligonucleotide followed by cytokine. The adhering cells are then added to the monolayer cells and incubated for 30 to 60 minutes at 37°C and washed to remove nonadhering cells. Cells adhering to the monolayer may be determined either by directly counting the adhering cells or prelabeling the cells with a radioisotope such as ^{51}Cr and quantitating the radioactivity associated with the monolayer as described. Dustin and Springer, *J. Cell Biol.*, 107:321-331 (1988). Antisense oligonucleotides may target either ICAM-1, VCAM-1 or ELAM-1 in the assay.

An example of the effects of antisense oligonucleotides targeting ICAM-1 mRNA on the adherence of DMSO differentiated HL-60 cells to tumor necrosis factor treated human umbilical vein endothelial cells is shown in Figure 7. Human umbilical vein endothelial cells were grown to 80% confluence in 12 well plates. The cells were treated with 2 μM oligonucleotide diluted in Opti-MEM containing 8 μM DOTMA for 4 hours at 37°C. The medium was removed and replaced with fresh endothelial cell growth medium (EGM-UV) containing 2 μM of the indicated oligonucleotide and incubated 4 hours at 37°C. Tumor necrosis factor, 1 ng/ml, was added to cells as indicated and cells incubated for an additional 19 hours. The cells were washed once with EGM-UV and 1.6 x 10^6 HL-60 cells differentiated for 4 days with 1.3% DMSO added. The cells were allowed to attach for 1 hour at 37°C and gently washed 4 times with Dulbecco's phosphate-buffered saline (D-PBS) warmed to 37°C. Adherent cells were detached from the monolayer by addition of 0.25 ml of cold (4°C) phosphate-buffered saline containing 5 mM EDTA and incubated on ice for 5 minutes. The number of cells removed by treatment with EDTA was determined by counting with a hemocytometer. Endothelial cells detached from the monolayer by EDTA treatment could easily be distinguished from HL-60 cells by morphological differences.

In the absence of tumor necrosis factor, 3% of the HL-60 cells bound to the endothelial cells. Treatment of the endothelial cell monolayer with 1 ng/ml tumor necrosis factor increased the number of adhering cells to 59% of total cells added (Figure 7). Treatment with the antisense oligonucleotide COMPOUND 2 or the control oligonucleotide COMPOUND 5 did not change the number of cells adhering to the monolayer in the absence of tumor necrosis factor treatment (Figure 7). The antisense oligonucleotide, COMPOUND 2 reduced the number of adhering cells from 59% of total cells added to 17% of the total cells added (Figure 7). In contrast, the control

oligonucleotide COMPOUND 5 did not significantly reduce the number of cells adhering to the tumor necrosis factor treated endothelial monolayer, i.e., 53% of total cells added for COMPOUND 5 treated cells versus 59% for control cells.

These data indicate that antisense oligonucleotides are capable of inhibiting ICAM-1 expression on endothelial cells and that inhibition of ICAM-1 expression correlates with a decrease in the adherence of a neutrophil-like cell to the endothelial monolayer in a sequence specific fashion. Because other molecules also mediate adherence of white blood cells to endothelial cells, such as ELAM-1, and VCAM-1 it is not expected that adherence would be completely blocked.

EXAMPLE 3

Synthesis and characterization of oligonucleotides and analogs:

Unmodified DNA oligonucleotides were synthesized on an automated DNA synthesizer (Applied Biosystems model 380B) using standard phosphoramidite chemistry with oxidation by iodine. ß-cyanoethyldiisopropyl-phosphoramidites were purchased from Applied Biosystems (Foster City, CA). For phosphorothioate oligonucleotides, the standard oxidation bottle was replaced by a 0.2 M solution of 3H-1,2-benzodithiole-3-one 1,1-dioxide in acetonitrile for the stepwise thiation of the phosphite linkages. The thiation cycle wait step was increased to 68 seconds and was followed by the capping step.

2'-O-methyl phosphorothioate oligonucleotides were synthesized using 2'-O-methyl ß-cyanoethyldiisopropyl-phosphoramidites (Chemgenes, Needham MA) and the standard cycle for unmodified oligonucleotides, except the wait step after pulse delivery of tetrazole and base was increased to 360 seconds. The 3'-base used to start the synthesis was a 2'-deoxyribonucleotide.

2'-fluoro phosphorothioate oligonucleotides were synthesized using 5'-dimethoxytrityl-3'-phosphoramidites and prepared as disclosed in U.S. patent application Serial

No. 463,358, filed January 11, 1990, and 566,977, filed August 13, 1990, which are assigned to the same assignee as the instant application and which are incorporated by reference herein. The 2'-fluoro oligonucleotides were prepared using phosphoramidite chemistry and a slight modification of the standard DNA synthesis protocol: deprotection was effected using methanolic ammonia at room temperature.

After cleavage from the controlled pore glass column (Applied Biosystems) and deblocking in concentrated ammonium hydroxide at 55°C for 18 hours, the oligonucleotides were purified by precipitation twice out of 0.5 M NaCl with 2.5 volumes ethanol. Analytical gel electrophoresis was accomplished in 20% acrylamide, 8 M urea, 45 mM Tris-borate buffer, pH 7.0. Oligodeoxynucleotides and their phosphorothioate analogs were judged from electrophoresis to be greater than 80% full length material.

RNA oligonucleotide synthesis was performed on an ABI model 380B DNA synthesizer. The standard synthesis cycle was modified by increasing the wait step after the pulse delivery of tetrazole to 900 seconds. The bases were deprotected by incubation in methanolic ammonia overnight. Following base deprotections the oligonucleotides were dried *in vacuo*. The t-butyldimethylsilyl protecting the 2' hydroxyl was removed by incubating the oligonucleotide in 1 M tetrabutylammoniumfluoride in tetrahydrofuran overnight. The RNA oligonucleotides were further purified on C_{18} Sep-Pak cartridges (Waters, Division of Millipore Corp., Milford MA) and ethanol precipitated.

The relative amounts of phosphorothioate and phosphodiester linkages obtained by this synthesis were periodically checked by ^{31}P NMR spectroscopy. The spectra were obtained at ambient temperature using deuterium oxide or dimethyl sulfoxide-d_6 as solvent. Phosphorothioate samples typically contained less than one percent of phosphodiester linkages.

Secondary evaluation was performed with oligonucleotides purified by trityl-on HPLC on a PRP-1 column (Hamilton Co., Reno, Nevada) using a gradient of acetonitrile in 50 mM triethylammonium acetate, pH 7.0 (4% to 32% in 30 minutes, flow rate = 1.5 ml/min). Appropriate fractions were pooled, evaporated and treated with 5% acetic acid at ambient temperature for 15 minutes. The solution was extracted with an equal volume of ethyl acetate, neutralized with ammonium hydroxide, frozen and lyophilized. HPLC-purified oligonucleotides were not significantly different in potency from precipitated oligonucleotides, as judged by the ELISA assay for ICAM-1 expression.

EXAMPLE 4

Cell culture and treatment with oligonucleotides:

The human lung carcinoma cell line A549 was obtained from the American Type Culture Collection (Bethesda MD). Cells were grown in Dulbecco's Modified Eagle's Medium (Irvine Scientific, Irvine CA) containing 1 gm glucose/liter and 10% fetal calf serum (Irvine Scientific). Human umbilical vein endothelial cells (HUVEC) (Clonetics, San Diego CA) were cultured in EGM-UV medium (Clonetics). HUVEC were used between the second and sixth passages. Human epidermal carcinoma A431 cells were obtained from the American Type Culture Collection and cultured in DMEM with 4.5 g/l glucose. Primary human keratinocytes were obtained from Clonetics and grown in KGM (Keratinocyte growth medium, Clonetics).

Cells grown in 96-well plates were washed three times with Opti-MEM (GIBCO, Grand Island, NY) prewarmed to 37°C. 100 μl of Opti-MEM containing either 10 μg/ml N-[1-(2,3-dioleyloxy)propyl]-N,N,N-trimethylammonium chloride (DOTMA, Bethesda Research Labs, Bethesda MD) in the case of HUVEC cells or 20 μg/ml DOTMA in the case of A549 cells was added to each well. Oligonucleotides were sterilized by centrifugation through 0.2 μm Centrex cellulose acetate filters (Schleicher and Schuell, Keene, NH).

Oligonucleotides were added as 20x stock solution to the wells and incubated for 4 hours at 37°C. Medium was removed and replaced with 150 µl of the appropriate growth medium containing the indicated concentration of oligonucleotide. Cells were incubated for an additional 3 to 4 hours at 37°C then stimulated with the appropriate cytokine for 14 to 16 hours, as indicated. ICAM-1 expression was determined as described in Example 1. The presence of DOTMA during the first 4 hours incubation with oligonucleotide increased the potency of the oligonucleotides at least 100-fold. This increase in potency correlated with an increase in cell uptake of the oligonucleotide.

EXAMPLE 5

ELISA screening of additional antisense oligonucleotides and oligonucleotide analogs for activity against ICAM-1 gene expression in Interleukin-1ß-stimulated cells:

Antisense oligonucleotides were originally designed that would hybridize to five target sites on the human ICAM-1 mRNA. Oligonucleotides were synthesized in both phosphodiester (P=O; ISIS 1558, 1559, 1563, 1564 and 1565) and phosphorothioate (P=S; ISIS 1570, 1571, 1572, 1573, and 1574) forms. The oligonucleotides and analogs are shown in Table 1.

TABLE 1

ANTISENSE OLIGONUCLEOTIDES WHICH TARGET HUMAN ICAM-1

ISIS NO.	SEQ ID NO.	TARGET REGION	MODIFICATION
1558	1	AUG Codon (64-81)	P=O
1559	2	5'-Untranslated (32049)	P=O
1563	3	3'-Untranslated (2190-3010)	P=O
1564	4	3'-Untranslated (2849-2866)	P=O
1565	5	Coding Region (1378-1395)	P=O
1570	1	AUG Codon (64-81)	P=S
1571	2	5'-Untranslated (32-49)	P=S
1572	3	3'-Untranslated (2190-3010)	P=S

	1573	4	3'-Untranslated (2849-2866)	P=S
	1574	5	Coding Region (1378-1395)	P=S
	1930	6	5'-Untranslated (1-20)	P=S
	1931	7	AUG Codon (55-74)	P=S
	1932	8	AUG Codon (72-91)	P=S
	1933	9	Coding Region (111-130)	P=S
	1934	10	Coding Region (351-370)	P=S
	1935	11	Coding Region (889-908)	P=S
	1936	12	Coding Region (1459-1468)	P=S
	1937	13	Termination Codon (1651-1687)	P=S
	1938	14	Termination Codon (1668-1687)	P=S
	1939	15	3'-Untranslated (1952-1971)	P=S
	1940	16	3'-Untranslated (2975-2994)	P=S
	2149	17	AUG Codon (64-77)	P=S
	2163	18	AUG Codon (64-75)	P=S
	2164	19	AUG Codon (64-73)	P=S
	2165	20	AUG Codon (66-80)	P=S
	2173	21	AUG Codon (64-79)	P=S
	2302	22	3'-Untranslated (2114-2133)	P=S
	2303	23	3'-Untranslated (2039-2058)	P=S
	2304	24	3'-Untranslated (1895-1914)	P=S
	2305	25	3'-Untranslated (1935-1954)	P=S
	2307	26	3'-Untranslated (1976-1995)	P=S
	2634	1	AUG-Codon (64-81)	2'-fluoro A,C & U; P=S
	2637	15	3'-Untranslated (1952-1971)	2'-fluoro A, C & U;

2691	1	AUG Codon (64-81)	P=O, except last 3 bases, P=S
2710	15	3'-Untranslated (1952-1971)	2'-O-methyl; P=O
2711	1	AUG Codon (64-81)	2'-O-methyl; P=O
2973	15	3'-Untranslated (1952-1971)	2'-O-methyl; P=S
2974	1	AUG Codon (64-81)	2'-O-methyl; P=S
3064	27	5'-CAP (32-51)	P=S; G & C added as spacer to 3'
3067	27	5'-CAP (32-51)	P=S
3222	27	5'-CAP (32-51)	2'-O-methyl; P=O
3224	27	5'-CAP (32-51)	2'-O-methyl; P=S

Inhibition of ICAM-1 expression on the surface of interleukin-1ß-stimulated cells by the oligonucleotides or oligonucleotide analogs was determined by ELISA assay as described in Example 1. The oligonucleotides were tested in two different cell lines. None of the phosphodiester oligonucleotides inhibited ICAM-1 expression. This is probably due to the rapid degradation of phosphodiester oligonucleotides in cells. Of the five phosphorothioate oligonucleotides, the most active was ISIS 1570, which hybridizes to the AUG translation initiation codon.

Based on the initial data obtained with the five original targets, twelve more oligonucleotides (ISIS 1930,

1940 and 3067, see Table 1) were designed which would hybridize with the ICAM-1 mRNA. The antisense oligonucleotide (ISIS 3067) which hybridizes to the predicted transcription initiation site (5' cap site) was approximately as active in IL-1ß-stimulated cells as the oligonucleotide that hybridizes to the AUG codon (ISIS 1570), as shown in Figure 8. ISIS 1931 and 1932 hybridize 5' and 3', respectively, to the AUG translation initiation codon. All three oligonucleotides that hybridize to the AUG region inhibit ICAM-1 expression, though ISIS 1932 was slightly less active than ISIS 1570 and ISIS 1931. Oligonucleotides which hybridize to the coding region of ICAM-1 mRNA (ISIS 1933, 1934, 1935, 1574 and 1936) exhibited weak activity. Oligonucleotides that hybridize to the translation termination codon (ISIS 1937 and 1938) exhibited moderate activity. Surprisingly, the most active antisense oligonucleotide, ISIS 1939, targeted a specific sequence in the 3'-untranslated region of the ICAM-1 mRNA. The antisense activity demonstrated by ISIS 1939 was not shared by other oligonucleotides (ISIS 1572, 1573, 1940) which hybridize to 3'-untranslated sequences. In fact, ISIS 1940, which targets the polyadenylation signal, failed to inhibit ICAM-1 expression.

Because ISIS 1939 proved unexpectedly to exhibit the greatest antisense activity of the original 16 oligonucleotides tested, other oligonucleotides were designed to hybridize to sequences in the 3'-untranslated region of ICAM-1 mRNA (ISIS 2302, 2303, 2304, 2305, and 2307, as shown in Table 1). ISIS 2307, which hybridizes to a site only five bases 3' to the ISIS 1939 target, was the least active of the series (Figure 8). ISIS 2302, which hybridizes to the ICAM-1 mRNA at a position 143 bases 3' to the ISIS 1939 target, was the most active of the series, with activity comparable to that of ISIS 1939. Examination of the predicted RNA secondary structure of the human ICAM-1 mRNA 3'-untranslated region (according to M. Zuker, *Science*, 244:48-52, 1989) revealed that both ISIS 1939 and

ISIS 2302 hybridize to sequences predicted to be in a stable stem-loop structure. Current dogma suggests that regions of RNA secondary structure should be avoided when designing antisense oligonucleotides. Thus, ISIS 1939 and ISIS 2302 would not have been predicted to inhibit ICAM-1 expression.

Several antisense oligonucleotide analogs containing modifications at the 2' position were also analyzed. The 2'-O-methyl phosphorothioate analog of ISIS 1570, ISIS 2974, was approximately threefold less effective than ISIS 1570 in inhibiting ICAM-1 expression in both HUVEC and A549 cells. In contrast, the 2'-O-methyl phosphorothioate analog of ISIS 1939 and ISIS 2973, failed to inhibit ICAM-1 expression in either cell line. Similar results were obtained with 2'-fluoro analogs of ISIS 1570, ISIS 1939 (ISIS 2634 and 2637, respectively).

The control oligonucleotide ISIS 1821 did inhibit ICAM-1 expression in HUVEC cells with activity comparable to that of ISIS 1934; however, in A549 cells ISIS 1821 was less effective than ISIS 1934. The negative control, ISIS 1821, was found to have a small amount of activity against ICAM expression, probably due in part to its ability to hybridize (12 of 13 base match) to the ICAM-1 mRNA at a position 15 bases 3' to the AUG translation initiation codon.

These studies indicate that the AUG translation initiation codon and specific 3'-untranslated sequences in the ICAM-1 mRNA were the most susceptible to antisense oligonucleotide inhibition of ICAM-1 expression.

In addition to inhibiting ICAM-1 expression in human umbilical vein cells and the human lung carcinoma cells (A549), ISIS 1570, ISIS 1939 and ISIS 2302 were shown to inhibit ICAM-1 expression in the human epidermal carcinoma A431 cells and in primary human keratinocytes (shown in Figure 9). These data demonstrate that antisense oligonucleotides are capable of inhibiting ICAM-1 expression in several human cell lines. Furthermore, the

rank order potency of the oligonucleotides is the same in the four cell lines examined. The fact that ICAM-1 expression could be inhibited in primary human keratinocytes is important because epidermal keratinocytes are an intended target of the antisense nucleotides.

EXAMPLE 6

Antisense oligonucleotide inhibition of ICAM-1 expression in cells stimulated with other cytokines:

Two oligonucleotides, ISIS 1570 and ISIS 1939, were tested for their ability to inhibit TNF-α and IFN-γ-induced ICAM-1 expression. Treatment of A549 cells with 1 uM antisense oligonucleotide inhibited IL-1ß, TNF-α and IFN-γ-induced ICAM-1 expression in a sequence-specific manner. The antisense oligonucleotides inhibited IL-1ß and TNF-α-induced ICAM-1 expression to a similar extent, while IFN-γ-induced ICAM-1 expression was more sensitive to antisense inhibition. The control oligonucleotide, ISIS 1821, did not significantly inhibit IL-1ß- or TNF-α-induced ICAM-1 expression and inhibited IFN-γ-induced ICAM-1 expression slightly, as follows:

Antisense Oligonucleotide (% Control Expression)

Cytokine	ISIS 1570	ISIS 1939	ISIS 1821
3 U/ml IL-1β	56.6 ± 2.9	38.1 ± 3.2	95 ± 6.6
1 ng/ml TNF-α	58.1 ± 0.9	37.6 ± 4.1	103.5 ± 8.2
100 U/ml gamma-IFN	38.9 ± 3.0	18.3 ± 7.0	83.0 ± 3.5

EXAMPLE 7

Antisense effects are abolished by sense strand controls:

The antisense oligonucleotide inhibition of ICAM-1 expression by the oligonucleotides ISIS 1570 and ISIS 1939 could be reversed by hybridization of the oligonucleotides with their respective sense strands. The phosphorothioate sense strand for ISIS 1570 (ISIS 1575), when applied alone, slightly enhanced IL-1ß-induced ICAM-1 expression. Premixing ISIS 1570 with ISIS 1575 at equal molar

concentrations, prior to addition to the cells, blocked the effects of ISIS 1570. The complement to ISIS 1939 (ISIS 2115) enhanced ICAM-1 expression by 46% when added to the cells alone. Prehybridization of ISIS 2115 to ISIS 1939 completely blocked the inhibition of ICAM-1 expression by ISIS 1939.

EXAMPLE 8

Measurement of oligonucleotide Tm (dissociation temperature of oligonucleotide from target):

To determine if the potency of the inhibition of ICAM-1 expression by antisense oligonucleotides was due to their affinity for their target sites, thermodynamic measurements were made for each of the oligonucleotides. The antisense oligonucleotides (synthesized as phosphorothioates) were hybridized to their complementary DNA sequences (synthesized as phosphodiesters). Absorbance vs. temperature profiles were measured at 4 μM each strand oligonucleotide in 100 mM Na+, 10 mM phosphate, 0.1 mM EDTA, pH 7.0. Tm's and free energies of duplex formation were obtained from fits of data to a two-state model with linear sloping baselines (Petersheim, M. and D.H. Turner (1983) *Biochemistry*, 22: 256-263). Results are averages of at least three experiments.

When the antisense oligonucleotides were hybridized to their complementary DNA sequences (synthesized as phosphodiesters), all of the antisense oligonucleotides with the exception of ISIS 1940 exhibited a Tm of at least 50°C. All the oligonucleotides should therefore be capable of hybridizing to the target ICAM-1 mRNA if the target sequences were exposed. Surprisingly, the potency of the antisense oligonucleotide did not correlate directly with either Tm or ΔG°_{37}. The oligonucleotide with the greatest biological activity, ISIS 1939, exhibited a Tm which was lower than that of the majority of the other oligonucleotides. Thus, hybridization affinity is not sufficient to ensure biological activity.

EXAMPLE 9

Effect of oligonucleotide length on antisense inhibition of ICAM-1 expression:

The effect of oligonucleotide length on antisense activity was tested using truncated versions of ISIS 1570 (ISIS 2165, 2173, 2149, 2163 and 2164) and ISIS 1939 (ISIS 2540, 2544, 2545, 2546, 2547 and 2548). In general, antisense activity decreased as the length of the oligonucleotides decreased. Oligonucleotides 16 bases in length exhibited activity slightly less than 18 base oligonucleotides. Oligonucleotides 14 bases in length exhibited significantly less activity, and oligonucleotides 12 or 10 bases in length exhibited only weak activity. Examination of the relationship between oligonucleotide length and Tm and antisense activity reveals that a sharp transition occurs between 14 and 16 bases in length, while Tm increases linearly with length (Figure 10).

EXAMPLE 10

Specificity of antisense inhibition of ICAM-1:

The specificity of the antisense oligonucleotides ISIS 1570 and ISIS 1939 for ICAM-1 was evaluated by immunoprecipitation of ^{35}S-labelled proteins. A549 cells were grown to confluence in 25 cm^2 tissue culture flasks and treated with antisense oligonucleotides as described in Example 4. The cells were stimulated with interleukin-1ß for 14 hours, washed with methionine-free DMEM plus 10% dialyzed fetal calf serum, and incubated for 1 hour in methionine-free medium containing 10% dialyzed fetal calf serum, 1 μM oligonucleotide and interleukin-1ß as indicated. ^{35}S-Methionine/cysteine mixture (Tran^{35}S-label, purchased from ICN, Costa Mesa, CA) was added to the cells to an activity of 100 μCi/ml and the cells were incubated an additional 2 hours. Cellular proteins were extracted by incubation with 50 mM Tris-HCl pH 8.0, 150 mM NaCl, 1.0% NP-40, 0.5% deoxycholate and 2mM EDTA (0.5 ml per well) at 4°C for 30 minutes. The extracts were clarified by centrifugation at 18,000 x g for 20 minutes. The supernatants were preadsorbed with 200 μl protein G-

Sepharose beads (Bethesda Research Labs, Bethesda MD) for 2 hours at 4°C, divided equally and incubated with either 5 μg ICAM-1 monoclonal antibody (purchased from AMAC Inc., Westbrook ME) or HLA-A,B antibody (W6/32, produced by murine hybridoma cells obtained from the American Type Culture Collection, Bethesda, MD) for 15 hours at 4°C. Immune complexes were trapped by incubation with 200 μl of a 50% suspension of protein G-Sepharose (v/v) for 2 hours at 4°C, washed 5 times with lysis buffer and resolved on an SDS-polyacrylamide gel. Proteins were detected by autoradiography.

Treatment of A549 cells with 5 units/ml of interleukin-1ß was shown to result in the synthesis of a 95-100 kDa protein migrating as a doublet which was immunoprecipitated with the monoclonal antibody to ICAM-1. The appearance as a doublet is believed to be due to differently glycosylated forms of ICAM-1. Pretreatment of the cells with the antisense oligonucleotide ISIS 1570 at a concentration of 1 μM decreased the synthesis of ICAM-1 by approximately 50%, while 1 μM ISIS 1939 decreased ICAM-1 synthesis to near background. Antisense oligonucleotide ISIS 1940, inactive in the ICAM-1 ELISA assay (Examples 1 and 5) did not significantly reduce ICAM-1 synthesis. None of the antisense oligonucleotides hybridizable with ICAM-1 targets had a demonstrable effect on HLA-A, B synthesis, demonstrating the specificity of the oligonucleotides for ICAM-1. Furthermore, the proteins which nonspecifically precipitated with the ICAM-1 antibody and protein G-Sepharose were not significantly affected by treatment with the antisense oligonucleotides.

EXAMPLE 11

Screening of additional antisense oligonucleotides and analogs for activity against ICAM-1 by cell adhesion assay:

Human umbilical vein endothelial (HUVEC) cells were grown and treated with oligonucleotides as in Example 4. Cells were treated with either antisense oligonucleotide ISIS 1939, the inactive antisense oligonucleotide ISIS 1940, or the control oligonucleotide

ISIS 1821 for 4 hours, then stimulated with TNF-α for 20 hours. Basal HUVEC minimally bound HL-60 cells, while TNF-stimulated HUVEC bound 19% of the total cells added. Pretreatment of the HUVEC monolayer with 0.3 μM ISIS 1939 reduced the adherence of HL-60 cells to basal levels, as shown in Figure 11. The control oligonucleotide, ISIS 1821 and the inactive oligonucleotide ISIS 1940 reduced the percentage of cells adhering from 19% to 9%. These data indicate that antisense oligonucleotides targeting ICAM-1 may specifically decrease adherence of a leukocyte-like cell line (HL-60) to TNF-α-treated HUVEC.

EXAMPLE 12

ELISA screening of additional antisense oligonucleotides and oligonucleotide analogs for activity against ELAM-1 gene expression:

Primary human umbilical vein endothelial (HUVEC) cells, passage 2 to 5, were plated in 96-well plates and allowed to reach confluence. Cells were washed three times with Opti-MEM (GIBCO, Grand Island NY). Cells were treated with increasing concentrations of oligonucleotide diluted in Opti-MEM containing 10 μg/ml DOTMA solution (Bethesda Research Labs, Bethesda MD) for 4 hours at 37°C. The medium was removed and replaced with EGM-UV (Clonetics, San Diego CA) plus oligonucleotide. Tumor necrosis factor α was added to the medium (2.5 ng/ml) and the cells were incubated an additional 4 hours at 37°C.

ELAM-1 expression was determined by ELISA. Cells were gently washed three times with Dulbecco's phosphate-buffered saline (D-PBS) prewarmed to 37°C. Cells were fixed with 95% ethanol at 4°C for 20 minutes, washed three times with D-PBS and blocked with 2% BSA in D-PBS. Cells were incubated with ELAM-1 monoclonal antibody BBA-1 (R&D Systems, Minneapolis MN) diluted to 0.5 μg/ml in D-PBS containing 2% BSA for 1 hour at 37°C. Cells were washed three times with D-PBS and the bound ELAM-1 antibody detected with biotinylated goat anti-mouse secondary antibody followed by ß-galactosidase-conjugated streptavidin as described in Example 1.

The activity of antisense phosphorothioate oligonucleotide analogs which target 11 different regions on the ELAM-1 cDNA and two oligonucleotides which target ICAM-1 (as controls) was determined using the ELAM-1 ELISA. The oligonucleotide analogs and targets are shown in Table 2.

TABLE 2

ANTISENSE OLIGONUCLEOTIDES WHICH TARGET HUMAN ELAM-1

ISIS NO.	SEQ ID NO.	TARGET REGION	MODIFICATION
1926	28	AUG Codon (143-164)	P=S
2670	29	3'-Untranslated (3718-3737)	P=S
2673	30	3'-Untranslated (2657-2677)	P=S
2674	31	3'-Untranslated (2617-2637)	P=S
2678	32	3'-Untranslated (3558-3577)	P=S
2679	33	5'-Untranslated (41-60)	P=S
2680	34	3'-Untranslated (3715-3729)	P=S
2683	35	AUG Codon (143-163)	P=S
2686	36	AUG Codon (149-169)	P=S
2687	37	5'-Untranslated (18-37)	P=S
2693	38	3'-Untranslated (2760-2788)	P=S
2694	39	3'-Untranslated (2934-2954)	P=S

In contrast to what was observed with antisense oligonucleotides targeted to ICAM-1 (Example 5), the most potent oligonucleotide modulator of ELAM-1 activity (ISIS 2679) was hybridizable with specific sequences in the 5'-untranslated region of ELAM-1. ISIS 2687, an oligonucleotide which hybridized to sequences ending three bases upstream of the ISIS 2679 target, did not show significant activity (Figure 12). Therefore, ISIS 2679 hybridizes to a unique site on the ELAM-1 mRNA, which is uniquely sensitive to inhibition with antisense

oligonucleotides. The sensitivity of this site to inhibition with antisense oligonucleotides was not predictable based upon RNA secondary structure predictions or information in the literature. Oligonucleotides which are hybridizable with the ELAM-1 AUG codon (ISIS 2683 and ISIS 2686) exhibit moderate activity (Figure 12).

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: Bennett et al.

(ii) TITLE OF INVENTION: Oligonucleotide Modulation of Cell Adhesion

(iii) NUMBER OF SEQUENCES: 39

(iv) CORRESPONDENCE ADDRESS:

(A) ADDRESSEE: Woodcock Washburn Kurtz Mackiewicz & Norris

(B) STREET: One Liberty Place - 46th Floor

(C) CITY: Philadelphia

(D) STATE: PA

(E) COUNTRY: USA

(F) ZIP: 19103

(v) COMPUTER READABLE FORM:

(A) MEDIUM TYPE: DISKETTE, 3.5 INCH, 1.44 Mb STORAGE

(B) COMPUTER: IBM PS/2

(C) OPERATING SYSTEM: PC-DOS

(D) SOFTWARE: WORDPERFECT 5.0

(vi) CURRENT APPLICATION DATA:

(A) APPLICATION NUMBER:

(B) FILING DATE:

(C) CLASSIFICATION:

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(A) APPLICATION NUMBER: 567,286

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(viii) ATTORNEY/AGENT INFORMATION:

(A) NAME: Jane Massey Licata

(B) REGISTRATION NUMBER: 32,257

(C) REFERENCE/DOCKET NUMBER: ISIS-0334

(ix) TELECOMMUNICATION INFORMATION:

(A) TELEPHONE: (215) 568-3100

(B) TELEFAX: (215) 568-3439

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 18

(B) TYPE: Nucleic Acid

(C) STRANDEDNESS: Single

(D) TOPOLOGY: Linear

(iv) ANTI-SENSE: Yes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

TGGGAGCCAT AGCGAGGC 18

(2) INFORMATION FOR SEQ ID NO: 2:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 20

(B) TYPE: Nucleic Acid

(C) STRANDEDNESS: Single

(D) TOPOLOGY: Linear

(iv) ANTI-SENSE: Yes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

GAGGAGCTCA GCGTCGACTG 20

(2) INFORMATION FOR SEQ ID NO: 3:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 21

(B) TYPE: Nucleic Acid

(C) STRANDEDNESS: Single

(D) TOPOLOGY: Linear

(iv) ANTI-SENSE: Yes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3:

GACACTCAAT AAATAGCTGG T 21

(2) INFORMATION FOR SEQ ID NO: 4:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 18

(B) TYPE: Nucleic Acid

(C) STRANDEDNESS: Single

(D) TOPOLOGY: Linear

(iv) ANTI-SENSE: Yes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4:

GAGGCTGAGG TGGGAGGA 18

(2) INFORMATION FOR SEQ ID NO: 5:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 18

(B) TYPE: Nucleic Acid

(C) STRANDEDNESS: Single

(D) TOPOLOGY: Linear

(iv) ANTI-SENSE: Yes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 5:

CGATGGGCAG TGGGAAAG 18

(2) INFORMATION FOR SEQ ID NO: 6:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 20

(B) TYPE: Nucleic Acid

(C) STRANDEDNESS: Single

(D) TOPOLOGY: Linear

(iv) ANTI-SENSE: Yes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 6:

GGGCGCGTGA TCCTTATAGC 20

(2) INFORMATION FOR SEQ ID NO: 7:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 20

(B) TYPE: Nucleic Acid

(C) STRANDEDNESS: Single

(D) TOPOLOGY: Linear

(iv) ANTI-SENSE: Yes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 7:

CATAGCGAGG CTGAGGTTGC 20

(2) INFORMATION FOR SEQ ID NO: 8:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 20

(B) TYPE: Nucleic Acid

(C) STRANDEDNESS: Single

(D) TOPOLOGY: Linear

(iv) ANTI-SENSE: Yes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 8:

CGGGGGCTGC TGGGAGCCAT 20

(2) INFORMATION FOR SEQ ID NO: 9:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 20

(B) TYPE: Nucleic Acid

(C) STRANDEDNESS: Single

(D) TOPOLOGY: Linear

(iv) ANTI-SENSE: Yes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 9:

AGAGCCCCGA GCAGGACCAG 20

(2) INFORMATION FOR SEQ ID NO: 10:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 20

(B) TYPE: Nucleic Acid

(C) STRANDEDNESS: Single

(D) TOPOLOGY: Linear

(iv) ANTI-SENSE: Yes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 10:

TGCCCATCAG GGCAGTTTGA 20

(2) INFORMATION FOR SEQ ID NO: 11:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 20

(B) TYPE: Nucleic Acid

(C) STRANDEDNESS: Single

(D) TOPOLOGY: Linear

(iv) ANTI-SENSE: Yes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 11:

GGTCACACTG ACTGAGGCCT 20

(2) INFORMATION FOR SEQ ID NO: 12:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 20

(B) TYPE: Nucleic Acid

(C) STRANDEDNESS: Single

(D) TOPOLOGY: Linear

(iv) ANTI-SENSE: Yes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 12:

CTCGCGGGTG ACCTCCCCTT 20

(2) INFORMATION FOR SEQ ID NO: 13:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 20

(B) TYPE: Nucleic Acid

(C) STRANDEDNESS: Single

(D) TOPOLOGY: Linear

(iv) ANTI-SENSE: Yes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 13:

TCAGGGAGGC GTGGCTTGTG 20

(2) INFORMATION FOR SEQ ID NO: 14:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 20

(B) TYPE: Nucleic Acid

(C) STRANDEDNESS: Single

(D) TOPOLOGY: Linear

(iv) ANTI-SENSE: Yes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 14:

CCTGTCCCGG GATAGGTTC A 20

(2) INFORMATION FOR SEQ ID NO: 15:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 20

(B) TYPE: Nucleic Acid

(C) STRANDEDNESS: Single

(D) TOPOLOGY: Linear

(iv) ANTI-SENSE: Yes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 15:

CCCCCACCAC TTCCCCTCTC 20

(2) INFORMATION FOR SEQ ID NO: 16:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 20

(B) TYPE: Nucleic Acid

(C) STRANDEDNESS: Single

(D) TOPOLOGY: Linear

(iv) ANTI-SENSE: Yes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 16:

TTGAGAAAGC TTTATTAACT 20

(2) INFORMATION FOR SEQ ID NO: 17:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 14

(B) TYPE: Nucleic Acid

(C) STRANDEDNESS: Single

(D) TOPOLOGY: Linear

(iv) ANTI-SENSE: Yes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 17:

AGCCATAGCG AGGC 14

(2) INFORMATION FOR SEQ ID NO: 18:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 12

(B) TYPE: Nucleic Acid

(C) STRANDEDNESS: Single

(D) TOPOLOGY: Linear

(iv) ANTI-SENSE: Yes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 18:

CCATAGCGAG GC 12

(2) INFORMATION FOR SEQ ID NO: 19:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 10

(B) TYPE: Nucleic Acid

(C) STRANDEDNESS: Single

(D) TOPOLOGY: Linear

(iv) ANTI-SENSE: Yes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 19:

ATAGCGAGGC 10

(2) INFORMATION FOR SEQ ID NO: 20:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 16

(B) TYPE: Nucleic Acid

(C) STRANDEDNESS: Single

(D) TOPOLOGY: Linear

(iv) ANTI-SENSE: Yes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 20:

TGGGAGCCAT AGCGAG 16

(2) INFORMATION FOR SEQ ID NO: 21:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 16

(B) TYPE: Nucleic Acid

(C) STRANDEDNESS: Single

(D) TOPOLOGY: Linear

(iv) ANTI-SENSE: Yes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 21:

GGAGCCATAG CGAGGC 16

(2) INFORMATION FOR SEQ ID NO: 22:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 20

(B) TYPE: Nucleic Acid

(C) STRANDEDNESS: Single

(D) TOPOLOGY: Linear

(iv) ANTI-SENSE: Yes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 22:

GCCCAAGCTG GCATCCGTCA 20

(2) INFORMATION FOR SEQ ID NO: 23:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 20

(B) TYPE: Nucleic Acid

(C) STRANDEDNESS: Single

(D) TOPOLOGY: Linear

(iv) ANTI-SENSE: Yes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 23:

TCTGTAAGTC TGTGGGCCTC 20

(2) INFORMATION FOR SEQ ID NO: 24:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 20

(B) TYPE: Nucleic Acid

(C) STRANDEDNESS: Single

(D) TOPOLOGY: Linear

(iv) ANTI-SENSE: Yes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 24:

AGTCTTGCTC CTTCCTCTTG 20

(2) INFORMATION FOR SEQ ID NO: 25:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 20

(B) TYPE: Nucleic Acid

(C) STRANDEDNESS: Single

(D) TOPOLOGY: Linear

(iv) ANTI-SENSE: Yes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 25:

CTCATCAGGC TAGACTTTAA 20

(2) INFORMATION FOR SEQ ID NO: 26:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 20

(B) TYPE: Nucleic Acid

(C) STRANDEDNESS: Single

(D) TOPOLOGY: Linear

(iv) ANTI-SENSE: Yes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 26:

TGTCCTCATG GTGGGGCTAT 20

(2) INFORMATION FOR SEQ ID NO: 27:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 22

(B) TYPE: Nucleic Acid

(C) STRANDEDNESS: Single

(D) TOPOLOGY: Linear

(iv) ANTI-SENSE: Yes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 27:

TCTGAGTAGC AGAGGAGCTC GA 22

(2) INFORMATION FOR SEQ ID NO: 28:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 22

(B) TYPE: Nucleic Acid

(C) STRANDEDNESS: Single

(D) TOPOLOGY: Linear

(iv) ANTI-SENSE: Yes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 28:

CAATCATGAC TTCAAGAGTT CT 22

(2) INFORMATION FOR SEQ ID NO: 29:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 20

(B) TYPE: Nucleic Acid

(C) STRANDEDNESS: Single

(D) TOPOLOGY: Linear

(iv) ANTI-SENSE: Yes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 29:

ACCACACTGG TATTTCACAC 20

(2) INFORMATION FOR SEQ ID NO: 30:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 21

(B) TYPE: Nucleic Acid

(C) STRANDEDNESS: Single

(D) TOPOLOGY: Linear

(iv) ANTI-SENSE: Yes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 30:

GTATGGAAGA TTATAATATA T 21

(2) INFORMATION FOR SEQ ID NO: 31:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 21

(B) TYPE: Nucleic Acid

(C) STRANDEDNESS: Single

(D) TOPOLOGY: Linear

(iv) ANTI-SENSE: Yes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 31:

CACAATCCTT AAGAACTCTT T 21

(2) INFORMATION FOR SEQ ID NO: 32:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 20

(B) TYPE: Nucleic Acid

(C) STRANDEDNESS: Single

(D) TOPOLOGY: Linear

(iv) ANTI-SENSE: Yes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 32:

ACCTCTGCTG TTCTGATCCT 20

(2) INFORMATION FOR SEQ ID NO: 33:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 20

(B) TYPE: Nucleic Acid

(C) STRANDEDNESS: Single

(D) TOPOLOGY: Linear

(iv) ANTI-SENSE: Yes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 33:

CTGCTGCCTC TGTCTCAGGT 20

(2) INFORMATION FOR SEQ ID NO: 34:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 15

(B) TYPE: Nucleic Acid

(C) STRANDEDNESS: Single

(D) TOPOLOGY: Linear

(iv) ANTI-SENSE: Yes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 34:

GGTATTTGAC ACAGC 15

(2) INFORMATION FOR SEQ ID NO: 35:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 21

(B) TYPE: Nucleic Acid

(C) STRANDEDNESS: Single

(D) TOPOLOGY: Linear

(iv) ANTI-SENSE: Yes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 35:

AATCATGACT TCAAGAGTTC T 21

(2) INFORMATION FOR SEQ ID NO: 36:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 21

(B) TYPE: Nucleic Acid

(C) STRANDEDNESS: Single

(D) TOPOLOGY: Linear

(iv) ANTI-SENSE: Yes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 36:

TGAAGCAATC ATGACTTCAA G 21

(2) INFORMATION FOR SEQ ID NO: 37:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 21

(B) TYPE: Nucleic Acid

(C) STRANDEDNESS: Single

(D) TOPOLOGY: Linear

(iv) ANTI-SENSE: Yes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 37:

TATAGGAGTT TTGATGTGAA 21

(2) INFORMATION FOR SEQ ID NO: 38:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 21

(B) TYPE: Nucleic Acid

(C) STRANDEDNESS: Single

(D) TOPOLOGY: Linear

(iv) ANTI-SENSE: Yes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 38:

ACAATGAGGG GGTAATCTAC A 21

(2) INFORMATION FOR SEQ ID NO: 39:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 21

(B) TYPE: Nucleic Acid

(C) STRANDEDNESS: Single

(D) TOPOLOGY: Linear

(iv) ANTI-SENSE: Yes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 39:

GACAATATAC AAACCTTCCA T 21

CLAIMS

What is claimed is:

1. An oligonucleotide or oligonucleotide analog specifically hybridizable with at least a portion of a nucleic acid encoding a protein capable of modulating cell adhesion.

2. The oligonucleotide or oligonucleotide analog of claim 1 which is specifically hybridizable with mRNA.

3. The oligonucleotide or oligonucleotide analog of claim 1 which is specifically hybridizable with a gene, forming a triple stranded structure for modulating the amount of mRNA made from said gene.

4. The oligonucleotide or oligonucleotide analog of claim 2 specifically hybridizable with a transcription initiation site, a translation initiation site, 5'-untranslated sequence, 3'-untranslated sequence, an intron/exon junction or an intervening sequence of mRNA.

5. The oligonucleotide or oligonucleotide analog of claim 4 specifically hybridizable with a 5' cap site or an adjacent sequence.

6. The oligonucleotide or oligonucleotide analog of claim 1 wherein said protein is intercellular adhesion molecule-1.

7. The oligonucleotide or oligonucleotide analog of claim 1 wherein said protein is endothelial leukocyte adhesion molecule-1.

8. The oligonucleotide or oligonucleotide analog of claim 1 wherein said protein is vascular cell adhesion molecule-1.

9. The oligonucleotide or oligonucleotide analog of claim 1 comprising from about 3 to about 50 subunits.

10. The oligonucleotide or oligonucleotide analog of claim 1 comprising from about 8 to about 25 subunits.

11. The oligonucleotide or oligonucleotide analog of claim 1 comprising from about 10 to about 20 subunits.

12. The oligonucleotide or oligonucleotide analog of claim 1 in a pharmaceutically acceptable carrier.

13. The oligonucleotide or oligonucleotide analog of claim 1 which is 2'-O-methyl, 2'-fluoro, phosphorothioate, 2'-O-methyl phosphorothioate, or 2'-fluoro phosphorothioate.

14. An oligonucleotide or oligonucleotide analog comprising one of the sequences identified in Table 1 or Table 2.

15. The oligonucleotide or oligonucleotide analog of claim 14 in a pharmaceutically acceptable carrier.

16. The oligonucleotide or oligonucleotide analog of claim 14 which is 2'-O-methyl, 2'-fluoro, phosphorothioate, 2'-O-methyl phosphorothioate, or 2'-fluoro phosphorothioate.

17. A method of modulating the synthesis of intercellular adhesion molecules in an animal comprising contacting the animal with an oligonucleotide or oligonucleotide analog specifically hybridizable with at least a portion of a nucleic acid encoding a protein capable of modulating cell adhesion.

18. The method of claim 17 wherein the oligonucleotide or oligonucleotide analog is specifically hybridizable with mRNA.

19. The method of claim 17 wherein the oligonucleotide or oligonucleotide analog is specifically hybridizable with a gene, forming a triple stranded structure for modulating the amount of mRNA made from said gene.

20. The method of claim 17 wherein the oligonucleotide or oligonucleotide analog is specifically hybridizable with a transcription initiation site, a translation initiation site, 5'-untranslated sequence, 3'

untranslated sequence, an intron/exon junction or an intervening sequence of mRNA.

21. The method of claim 17 wherein said oligonucleotide or oligonucleotide analog is specifically hybridizable with a 5' cap site of mRNA or an adjacent sequence.

22. The method of claim 17 wherein said protein is intercellular adhesion molecule-1.

23. The method of claim 17 wherein said protein is endothelial leukocyte adhesion molecule-1.

24. The method of claim 17 wherein said protein is vascular cell adhesion molecule-1.

25. The method of claim 17 wherein the oligonucleotide or oligonucleotide analog comprises a sequence identified in Table 1 or Table 2.

26. The method of claim 17 wherein said oligonucleotide or oligonucleotide analog is 2'-O-methyl, 2'-fluoro, phosphorothioate, 2'-O-methyl phosphorothioate, or 2'-fluoro phosphorothioate.

27. A method of treating an animal suspected of having a disease which is modulated by changes in intercellular adhesion molecules comprising contacting the animal with an oligonucleotide or oligonucleotide analog specifically hybridizable with nucleic acids encoding at least a portion of a protein which modulates the synthesis or metabolism of intercellular adhesion molecules.

28. The method of claim 27 wherein said oligonucleotide or oligonucleotide analog is specifically hybridizable to a transcription initiation site, translation initiation site, 5'-untranslated sequences, 3' untranslated sequences or an intervening sequence of mRNA.

29. The method of claim 27 wherein said oligonucleotide or oligonucleotide analog is specifically hybridizable with a 5' cap site of mRNA and adjacent sequences.

30. The method of claim 27 wherein said protein is intercellular adhesion molecule-1.

31. The method of claim 27 wherein said protein is endothelial leukocyte adhesion molecule-1.

32. The method of claim 27 wherein said protein is vascular cell adhesion molecule-1.

33. The method of claim 27 wherein the oligonucleotide or oligonucleotide analog is in a pharmaceutically acceptable carrier.

34. The method of claim 27 wherein the oligonucleotide or oligonucleotide analog comprises one of the sequences identified in Table 1 or Table 2.

35. The method of claim 27 wherein said oligonucleotide or oligonucleotide analog is 2'-O-methyl, 2'-fluoro, phosphorothioate, 2'-O-methyl phosphorothioate, or 2'-fluoro phosphorothioate.

FIGURE 1

HUMAN ICAM-1 cDNA SEQUENCE

```
1:       GCTATAAGGA TCACGCGCCC CAGTCGACGC TGAGCTCCTC TGCTACTCAG AGTTGCAACC TCAGCCTCGC T ATG GCT CCC
                                                                                    MET ALA PRO
81:  AGC AGC CCC CGG CCC GCG CTG CCC GCA CTC CTG GTC CTG CTC GGG GCT CTG TTC CCA GGA CCT GGC A
     SER SER PRO ARG PRO ALA LEU PRO ALA LEU LEU VAL LEU LEU GLY ALA LEU PHE PRO GLY PRO GLY A

148: AT GCC CAG ACA TCT GTG TCC CCC TCA AAA GTC ATC CTG CCC CGG GGA GGC TCC GTG CTG GTG ACA TGC
     SN ALA GLN THR SER VAL SER PRO SER LYS VAL ILE LEU PRO ARG GLY GLY SER VAL LEU VAL THR CYS

216:  AGC ACC TCC TGT GAC CAG CCC AAG TTG TTG GGC ATA GAG ACC CCG TTG CCT AAA AAG GAG TTG CTC C
      SER THR SER CYS ASP GLN PRO LYS LEU LEU GLY ILE GLU THR PRO LEU PRO LYS LYS GLU LEU LEU L

283: TG CCT GGG AAC AAC CGG AAG GTG TAT GAA CTG AGC AAT GTG CAA GAA GAT AGC CAA CCA ATG TGC TAT
     EU PRO GLY ASN ASN ARG LYS VAL TYR GLU LEU SER ASN VAL GLN GLU ASP SER GLN PRO MET CYS TYR

351:  TCA AAC TGC CCT GAT GGG CAG TCA ACA GCT AAA ACC TTC CTC ACC GTG TAC TGG ACT CCA GAA CGG G
      SER ASN CYS PRO ASP GLY GLN SER THR ALA LYS THR PHE LEU THR VAL TYR TRP THR PRO GLU ARG V

418: TG GAA CTG GCA CCC CTC CCC TCT TGG CAG CCA GTG GGC AAG AAC CTT ACC CTA CGC TGC CAG GTG GAG
     AL GLU LEU ALA PRO LEU PRO SER TRP GLN PRO VAL GLY LYS ASN LEU THR LEU ARG CYS GLN VAL GLU

486:  GGT GGG GCA CCC CGG GCC AAC CTC ACC GTG GTG CTG CTC CGT GGG GAG AAG GAG CTG AAA CGG GAG C
      GLY GLY ALA PRO ARG ALA ASN LEU THR VAL VAL LEU LEU ARG GLY GLU LYS GLU LEU LYS ARG GLU P

553: CA GCT GTG GGG GAG CCC GCT GAG GTC ACG ACC ACG GTG CTG GTG AGG AGA GAT CAC CAT GGA GCC AAT
     RO ALA VAL GLY GLU PRO ALA GLU VAL THR THR THR VAL LEU VAL ARG ARG ASP HIS HIS GLY ALA ASN

621:  TTC TCG TGC CGC ACT GAA CTG GAC CTG CGG CCC CAA GGG CTG GAG CTG TTT GAG AAC ACC TCG GCC C
      PHE SER CYS ARG THR GLU LEU ASP LEU ARG PRO GLN GLY LEU GLU LEU PHE GLU ASN THR SER ALA P

688: CC TAC CAG CTC CAG ACC TTT GTC CTG CCA GCG ACT CCC CCA CAA CTT GTC AGC CCC CGG GTC CTA GAG
     RO TYR GLN LEU GLN THR PHE VAL LEU PRO ALA THR PRO PRO GLN LEU VAL SER PRO ARG VAL LEU GLU

756:  GTG GAC ACG CAG GGG ACC GTG GTC TGT TCC CTG GAC GGG CTG TTC CCA GTC TCG GAG GCC CAG GTC C
      VAL ASP THR GLN GLY THR VAL VAL CYS SER LEU ASP GLY LEU PHE PRO VAL SER GLU ALA GLN VAL H

823: AC CTG GCA CTG GGG GAC CAG AGG TTG AAC CCC ACA GTC ACC TAT GGC AAC GAC TCC TTC TCG GCC AAG
     IS LEU ALA LEU GLY ASP GLN ARG LEU ASN PRO THR VAL THR TYR GLY ASN ASP SER PHE SER ALA LYS

891:  GCC TCA GTC AGT GTG ACC GCA GAG GAC GAG GGC ACC CAG CGG CTG ACG TGT GCA GTA ATA CTG GGG A
      ALA SER VAL SER VAL THR ALA GLU ASP GLU GLY THR GLN ARG LEU THR CYS ALA VAL ILE LEU GLY A

958: AC CAG AGC CAG GAG ACA CTG CAG ACA GTG ACC ATC TAC AGC TTT CCG GCG CCC AAC GTG ATT CTG ACG
     SN GLN SER GLN GLU THR LEU GLN THR VAL THR ILE TYR SER PHE PRO ALA PRO ASN VAL ILE LEU THR

1026:  AAG CCA GAG GTC TCA GAA GGG ACC GAG GTG ACA GTG AAG TGT GAG GCC CAC CCT AGA GCC AAG GTG A
       LYS PRO GLU VAL SER GLU GLY THR GLU VAL THR VAL LYS CYS GLU ALA HIS PRO ARG ALA LYS VAL T

1093: CG CTG AAT GGG GTT CCA GCC CAG CCA CTG GGC CCG AGG GCC CAG CTC CTG CTG AAG GCC ACC CCA GAG
      HR LEU ASN GLY VAL PRO ALA GLN PRO LEU GLY PRO ARG ALA GLN LEU LEU LEU LYS ALA THR PRO GLU

1161:  GAC AAC GGG CGC AGC TTC TCC TGC TCT GCA ACC CTG GAG GTG GCC GGC CAG CTT ATA CAC AAG AAC C
       ASP ASN GLY ARG SER PHE SER CYS SER ALA THR LEU GLU VAL ALA GLY GLN LEU ILE HIS LYS ASN G

1228: AG ACC CGG GAG CTT CGT GTC CTG TAT GGC CCC CGA CTG GAC GAG AGG GAT TGT CCG GGA AAC TGG ACG
      LN THR ARG GLU LEU ARG VAL LEU TYR GLY PRO ARG LEU ASP GLU ARG ASP CYS PRO GLY ASN TRP THR

1296:  TGG CCA GAA AAT TCC CAG CAG ACT CCA ATG TGC CAG GCT TGG GGG AAC CCA TTG CCC GAG CTC AAG T
       TRP PRO GLU ASN SER GLN GLN THR PRO MET CYS GLN ALA TRP GLY ASN PRO LEU PRO GLU LEU LYS C

1363: GT CTA AAG GAT GGC ACT TTC CCA CTG CCC ATC GGG GAA TCA GTG ACT GTC ACT CGA GAT CTT GAG GGC
      YS LEU LYS ASP GLY THR PHE PRO LEU PRO ILE GLY GLU SER VAL THR VAL THR ARG ASP LEU GLU GLY

1431:  ACC TAC CTC TGT CGG GCC AGG AGC ACT CAA GGG GAG GTC ACC CGC GAG GTG ACC GTG AAT GTG CTC T
       THR TYR LEU CYS ARG ALA ARG SER THR GLN GLY GLU VAL THR ARG GLU VAL THR VAL ASN VAL LEU S

1498: CC CCC CGG TAT GAG ATT GTC ATC ATC ACT GTG GTA GCA GCC GCA GTC ATA ATG GGC ACT GCA GGC CTC
      ER PRO ARG TYR GLU ILE VAL ILE ILE THR VAL VAL ALA ALA ALA VAL ILE MET GLY THR ALA GLY LEU

1566:  AGC ACG TAC CTC TAT AAC CGC CAG CGG AAG ATC AAG AAA TAC AGA CTA CAA CAG GCC CAA AAA GGG A
       SER THR TYR LEU TYR ASN ARG GLN ARG LYS ILE LYS LYS TYR ARG LEU GLN GLN ALA GLN LYS GLY T

1633: CC CCC ATG AAA CCG AAC ACA CAA GCC ACG CCT CCC TGA ACCTATCCCG GGACAGGGCC TCTTCCTCGG CCTTCC
      HR PRO MET LYS PRO ASN THR GLN ALA THR PRO PRO ***

1707: CATA TTGGTGGCAG TGGTGCCACA CTGAACAGAG TGGAAGACAT ATGCCATGCA GCTACACCTA CCGGCCCTGG GACGCCGG
```

1789: AG GACAGGGCAT TGTCCTCAGT CAGATACAAC AGCATTTGGG GCCATGGTAC CTGCACACCT AAAACACTAG GCCACGCATC

1871: TGATCTGTAG TCACATGACT AAGCCAAGAG GAAGGAGCAA GACTCAAGAC ATGATTGATG GATGTTAAAG TCTAGCCTGA T

1952: GAGAGGGGA AGTGGTGGGG GAGACATAGC CCCACCATGA GGACATACAA CTGGGAAATA CTGAAACTTG CTGCCTATTG GGT

2034: ATGCTGA GGCCCACAGA CTTACAGAAG AAGTGGCCCT CCATAGACAT GTGTAGCATC AAAACACAAA GGCCCACACT TCCTG

2116: ACGGA TGCCAGCTTG GGCACTGCTG TCTACTGACC CCAACCCTTG ATGATATGTA TTTATTCATT TGTTATTTTA CCAGCTA

2198: TTT ATTGAGTGTC TTTTATGTAG GCTAAATGAA CATAGGTCTC TGGCCTCACG GAGCTCCCAG TCCATGTCAC ATTCAAGGT

2280: C ACCAGGTACA GTTGTACAGG TTGTACACTG CAGGAGAGTG CCTGGCAAAA AGATCAAATG GGGCTGGGAC TTCTCATTGG

2361: CCAACCTGCC TTTCCCCAGA AGGAGTGATT TTTCTATCGG CACAAAAGCA CTATATGGAC TGGTAATGGT TCACAGGTTC AG

2443: AGATTACC CAGTGAGGCC TTATTCCTCC CTTCCCCCCA AAACTGACAC CTTTGTTAGC CACCTCCCCA CCCACATACA TTTC

2525: TGCCAG TGTTACAATG ACACTCAGCG GTCATGTCTG GACATGAGTG CCCAGGGAAT ATGCCCAAGC TATGCCTTGT CCTCTT

2607: GTCC TGTTTGCATT TCACTGGGAG CTTGCACTAT TGCAGCTCCA GTTTCCTGCA GTGATCAGGG TCCTGCAAGC AGTGGGGA

2689: AG GGGGCCAAGG TATTGGAGGA CTCCCTCCCA GCTTTGGAAG GGTCATCCGC GTGTGTGTGT GTGTGTATGT GTAGACAAGC

2771: TCTCGCTCTG TCACCCAGGC TGGAGTGCAG TGGTGCAATC ATGGTTCACT GCAGTCTTGA CCTTTTGGGC TCAAGTGATC C

2852: TCCCACCTC AGCCTCCTGA GTAGCTGGGA CCATAGGCTC ACAACACCAC ACCTGGCAAA TTTGATTTTT TTTTTTTTT TCA

2934: GAGACGG GGTCTCGCAA CATTGCCCAG ACTTCCTTTG TGTTAGTTAA TAAAGCTTTC TCAACTGCC AAAAAAAAAAAAAAAAA

FIGURE 2

HUMAN ELAM-1 mRNA SEQUENCE

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   1: TTCACATCAA AACTCCTATA CTGACCTGAG ACAGAGGCAG CAGTGATACC CACCTGAGAG ATCCTGTGTT TGA

  74: ACAACTG CTTCCCAAAA CGGAAAGTAT TTCAAGCCTA AACCTTTGGG TGAAAAGAAC TCTTGAAGTC ATG AT
                                                                              met il

 146: T GCT TCA CAG TTT CTC TCA GCT CTC ACT TTG GTG CTT CTC ATT AAA GAG AGT GGA GCC TG
      e ala ser gln phe leu ser ala leu thr leu val leu leu ile lys glu ser gly ala tr

 206: G TCT TAC AAC ACC TCC ACG GAA GCT ATG ACT TAT GAT GAG GCC AGT GCT TAT TGT CAG CA
      p ser tyr asn thr ser thr glu ala met thr tyr asp glu ala ser ala tyr cys gln gl

 266: A AGG TAC ACA CAC CTG GTT GCA ATT CAA AAC AAA GAA GAG ATT GAG TAC CTA AAC TCC AT
      n arg tyr thr his leu val ala ile gln asn lys glu glu ile glu tyr leu asn ser il

 326: A TTG AGC TAT TCA CCA AGT TAT TAC TGG ATT GGA ATC AGA AAA GTC AAC AAT GTG TGG GT
      e leu ser tyr ser pro ser tyr tyr trp ile gly ile arg lys val asn asn val trp va

 386: C TGG GTA GGA ACC CAG AAA CCT CTG ACA GAA GAA GCC AAG AAC TGG GCT CCA GGT GAA CC
      l trp val gly thr gln lys pro leu thr glu glu ala lys asn trp ala pro gly glu pr

 446: C AAC AAT AGG CAA AAA GAT GAG GAC TGC GTG GAG ATC TAC ATC AAG AGA GAA AAA GAT GT
      o asn asn arg gln lys asp glu asp cys val glu ile tyr ile lys arg glu lys asp va

 506: G GGC ATG TGG AAT GAT GAG AGG TGC AGC AAG AAG AAG CTT GCC CTA TGC TAC ACA GCT GC
      l gly met trp asn asp glu arg cys ser lys lys lys leu ala leu cys tyr thr ala al

 566: C TGT ACC AAT ACA TCC TGC AGT GGC CAC GGT GAA TGT GTA GAG ACC ATC AAT AAT TAC AC
      a cys thr asn thr ser cys ser gly his gly glu cys val glu thr ile asn asn tyr th

 626: T TGC AAG TGT GAC CCT GGC TTC AGT GGA CTC AAG TGT GAG CAA ATT GTG AAC TGT ACA GC
      r cys lys cys asp pro gly phe ser gly leu lys cys glu gln ile val asn cys thr al

 686: C CTG GAA TCC CCT GAG CAT GGA AGC CTG GTT TGC AGT CAC CCA CTG GGA AAC TTC AGC TA
      a leu glu ser pro glu his gly ser leu val cys ser his pro leu gly asn phe ser ty

 746: C AAT TCT TCC TGC TCT ATC AGC TGT GAT AGG GGT TAC CTG CCA AGC AGC ATG GAG ACC AT
      r asn ser ser cys ser ile ser cys asp arg gly tyr leu pro ser ser met glu thr me

 806: G CAG TGT ATG TCC TCT GGA GAA TGG AGT GCT CCT ATT CCA GCC TGC AAT GTG GTT GAG TG
      t gln cys met ser ser gly glu trp ser ala pro ile pro ala cys asn val val glu cy

 866: T GAT GCT GTG ACA AAT CCA GCC AAT GGG TTC GTG GAA TGT TTC CAA AAC CCT GGA AGC TT
      s asp ala val thr asn pro ala asn gly phe val glu cys phe gln asn pro gly ser ph

 926: C CCA TGG AAC ACA ACC TGT ACA TTT GAC TGT GAA GAA GGA TTT GAA CTA ATG GGA GCC CA
      e pro trp asn thr thr cys thr phe asp cys glu glu gly phe glu leu met gly ala gl

 986: G AGC CTT CAG TGT ACC TCA TCT GGG AAT TGG GAC AAC GAG AAG CCA ACG TGT AAA GCT GT
      n ser leu gln cys thr ser ser gly asn trp asp asn glu lys pro thr cys lys ala va

1046: G ACA TGC AGG GCC GTC CGC CAG CCT CAG AAT GGC TCT GTG AGG TGC AGC CAT TCC CCT GC
      l thr cys arg ala val arg gln pro gln asn gly ser val arg cys ser his ser pro al

1106: T GGA GAG TTC ACC TTC AAA TCA TCC TGC AAC TTC ACC TGT GAG GAA GGC TTC ATG TTG CA
      a gly glu phe thr phe lys ser ser cys asn phe thr cys glu glu gly phe met leu gl

1166: G GGA CCA GCC CAG GTT GAA TGC ACC ACT CAA GGG CAG TGG ACA CAG CAA ATC CCA GTT TG
      n gly pro ala gln val glu cys thr thr gln gly gln trp thr gln gln ile pro val cy

1226: T GAA GCT TTC CAG TGC ACA GCC TTG TCC AAC CCC GAG CGA GGC TAC ATG AAT TGT CTT CC
      s glu ala phe gln cys thr ala leu ser asn pro glu arg gly tyr met asn cys leu pr

1286: T AGT GCT TCT GGC AGT TTC CGT TAT GGG TCC AGC TGT GAG TTC TCC TGT GAG CAG GGT TT
      o ser ala ser gly ser phe arg tyr gly ser ser cys glu phe ser cys glu gln gly ph
```

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1346: T GTG TTG AAG GGA TCC AAA AGG CTC CAA TGT GGC CCC ACA GGG GAG TGG GAC AAC GAG AA
      e val leu lys gly ser lys arg leu gln cys gly pro thr gly glu trp asp asn glu ly

1406: G CCC ACA TGT GAA GCT GTG AGA TGC GAT GCT GTC CAC CAG CCC CCG AAG GGT TTG GTG AG
      s pro thr cys glu ala val arg cys asp ala val his gln pro pro lys gly leu val ar

1466: G TGT GCT CAT TCC CCT ATT GGA GAA TTC ACC TAC AAG TCC TCT TGT GCC TTC AGC TGT GA
      g cys ala his ser pro ile gly glu phe thr tyr lys ser ser cys ala phe ser cys gl

1526: G GAG GGA TTT GAA TTA TAT GGA TCA ACT CAA CTT GAG TGC ACA TCT CAG GGA CAA TGG AC
      u glu gly phe glu leu tyr gly ser thr gln leu glu cys thr ser gln gly gln trp th

1586: A GAA GAG GTT CCT TCC TGC CAA GTG GTA AAA TGT TCA AGC CTG GCA GTT CCG GGA AAG AT
      r glu glu val pro ser cys gln val val lys cys ser ser leu ala val pro gly lys il

1646: C AAC ATG AGC TGC AGT GGG GAG CCC GTG TTT GGC ACT GTG TGC AAG TTC GCC TGT CCT GA
      e asn met ser cys ser gly glu pro val phe gly thr val cys lys phe ala cys pro gl

1706: A GGA TGG ACG CTC AAT GGC TCT GCA GCT CGG ACA TGT GGA GCC ACA GGA CAC TGG TCT GG
      u gly trp thr leu asn gly ser ala ala arg thr cys gly ala thr gly his trp ser gl

1766: C CTG CTA CCT ACC TGT GAA GCT CCC ACT GAG TCC AAC ATT CCC TTG GTA GCT GGA CTT TC
      y leu leu pro thr cys glu ala pro thr glu ser asn ile pro leu val ala gly leu se

1826: T GCT GCT GGA CTC TCC CTC CTG ACA TTA GCA CCA TTT CTC CTC TGG CTT CGG AAA TGC TT
      r ala ala gly leu ser leu leu thr leu ala pro phe leu leu trp leu arg lys cys le

1886: A CGG AAA GCA AAG AAA TTT GTT CCT GCC AGC AGC TGC CAA AGC CTT GAA TCA GAC GGA AG
      u arg lys ala lys lys phe val pro ala ser ser cys gln ser leu glu ser asp gly se

1946: C TAC CAA AAG CCT TCT TAC ATC CTT TAA GTTCAAA AGAATCAGAA ACAGGTGCAT CTGGGGAACT A
      r tyr gln lys pro ser tyr ile leu ***

2012: GAGGGATAC ACTGAAGTTA ACAGAGACAG ATAACTCTCC TCGGGTCTCT GGCCCTTCTT GCCTACTATG CCAG

2085: ATGCCT TTATGGCTGA AACCGCAACA CCCATCACCA CTTCAATAGA TCAAAGTCCA GCAGGCAAGG ACGGCCT

2158: TCA ACTGAAAAGA CTCAGTGTTC CCTTTCCTAC TCTCAGGATC AAGAAAGTGT TGGCTAATGA AGGGAAAGGA

2231:  TATTTTCTTC CAAGCAAAGG TGAAGAGACC AAGACTCTGA AATCTCAGAA TTCCTTTTCT AACTCTCCCT TG

2303: CTCGCTGT AAAATCTTGG CACAGAAACA CAATATTTTG TGGCTTTCTT TCTTTTGCCC TTCACAGTGT TTCGA

2376: CAGCT GATTACACAG TTGCTGTCAT AAGAATGAAT AATAATTATC CAGAGTTTAG AGGAAAAAAA TGACTAAA

2449: AA TATTATAACT TAAAAAAATG ACAGATGTTG AATGCCCACA GGCAAATGCA TGGAGGGTTG TTAATGGTGC

2521: AAATCCTACT GAATGCTCTG TGCGAGGGTT ACTATGCACA ATTTAATCAC TTTCATCCCT ATGGGATTCA GTG

2594: CTTCTTA AAGAGTTCTT AAGGATTGTG ATATTTTTAC TTGCATTGAA TATATTATAA TCTTCCATAC TTCTTC

2667: ATTC AATACAAGTG TGGTAGGGAC TTAAAAAACT TGTAAATGCT GTCAACTATG ATATGGTAAA AGTTACTTA

2740: T TCTAGATTAC CCCCTCATTG TTTATTAACA AATTATGTTA CATCTGTTTT AAATTTATTT CAAAAAGGGA A

2812: ACTATTGTC CCCTAGCAAG GCATGATGTT AACCAGAATA AAGTTCTGAG TGTTTTTACT ACAGTTGTTT TTTG

2885: AAAACA TGGTAGAATT GGAGAGTAAA AACTGAATGG AAGGTTTGTA TATTGTCAGA TATTTTTTCA GAAATAT

2958: GTG GTTTCCACGA TGAAAAACTT CCATGAGGCC AAACGTTTTG AACTAATAAA AGCATAAATG CAAACACACA

3031:  AAGGTATAAT TTTATGAATG TCTTTGTTGG AAAAGAATAC AGAAAGATGG ATGTGCTTTG CATTCCTACA AA

3103: GATGTTTG TCAGATGTGA TATGTAAACA TAATTCTTGT ATATTATGGA AGATTTTAAA TTCACAATAG AAACT

3176: CACCA TGTAAAAGAG TCATCTGGTA GATTTTTAAC GAATGAAGAT GTCTAATAGT TATTCCCTAT TTGTTTTC

3249: TT CTGTATGTTA GGGTGCTCTG GAAGAGAGGA ATGCCTGTGT GAGCAAGCAT TTATGTTTAT TTATAAGCAG

3321: ATTTAACAAT TCCAAAGGAA TCTCCAGTTT TCAGTTGATC ACTGGCAATG AAAAATTCTC AGTCAGTAAT TGC

3394: CAAAGCT GCTCTAGCCT TGAGGAGTGT GAGAATCAAA ACTCTCCTAC ACTTCCATTA ACTTAGCATG TGTTGA
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3467: AAAA AAAAGTTTCA GAGAAGTTCT GGCTGAACAC TGGCAACGAC AAAGCCAACA GTCAAAACAG AGATGTGAT

3540: A AGGATCAGAA CAGCAGAGGT TCTTTTAAAG GGGCAGAAAA ACTCTGGGAA ATAAGAGAGA ACAACTACTG T

3612: GATCAGGCT ATGTATGGAA TACAGTGTTA TTTTCTTTGA AATTGTTTAA GTGTTGTAAA TATTTATGTA AACT

3685: GCATTA GAAATTAGCT GTGTGAAATA CCAGTGTGGT TTGTGTTTGA GTTTTATTGA GAATTTTAAA TTATAAC

3758: TTA AAATATTTTA TAATTTTTAA AGTATATATT TATTTAAGCT TATGTCAGAC CTATTTGACA TAACACTATA

3831: AAGGTTGACA ATAAATGTGC TTATGTTT

FIGURE 3

HUMAN VCAM-1 mRNA SEQUENCE

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   1: CGGGCCTCAC TGGCTTCAGG AGCTGAATAC CCTCCCAGGC ACACACAGGT GGGACACAAA TAAGGGTTTT GGA

  74: ACCACTA TTTTCTCATC ACGACAGCAA CTTAAA ATG CCT GGG AAG ATG GTC GTG ATC CTT GGA GCC
                                          met pro gly lys met val val ile leu gly ala

 140:  TCA AAT ATA CTT TGG ATA ATG TTT GCA GCT TCT CAA GCT TTT AAA ATC GAG ACC ACC CCA
       ser asn ile leu trp ile met phe ala ala ser gln ala phe lys ile glu thr thr pro

 200:  GAA TCT AGA TAT CTT GCT CAG ATT GGT GAC TCC GTC TCA TTG ACT TGC AGC ACC ACA GGC
       glu ser arg tyr leu ala gln ile gly asp ser val ser leu thr cys ser thr thr gly

 260:  TGT GAG TCC CCA TTT TTC TCT TGG AGA ACC CAG ATA GAT AGT CCA CTG AAT GGG AAG GTG
       cys glu ser pro phe phe ser trp arg thr gln ile asp ser pro leu asn gly lys val

 320:  ACG AAT GAG GGG ACC ACA TCT ACG CTG ACA ATG AAT CCT GTT AGT TTT GGG AAC GAA CAC
       thr asn glu gly thr thr ser thr leu thr met asn pro val ser phe gly asn glu his

 380:  TCT TAC CTG TGC ACA GCA ACT TGT GAA TCT AGG AAA TTG GAA AAA GGA ATC CAG GTG GAG
       ser tyr leu cys thr ala thr cys glu ser arg lys leu glu lys gly ile gln val glu

 440:  ATC TAC TCT TTT CCT AAG GAT CCA GAG ATT CAT TTG AGT GGC CCT CTG GAG GCT GGG AAG
       ile tyr ser phe pro lys asp pro glu ile his leu ser gly pro leu glu ala gly lys

 500:  CCG ATC ACA GTC AAG TGT TCA GTT GCT GAT GTA TAC CCA TTT GAC AGG CTG GAG ATA GAC
       pro ile thr val lys cys ser val ala asp val tyr pro phe asp arg leu glu ile asp

 560:  TTA CTG AAA GGA GAT CAT CTC ATG AAG AGT CAG GAA TTT CTG GAG GAT GCA GAC AGG AAG
       leu leu lys gly asp his leu met lys ser gln glu phe leu glu asp ala asp arg lys

 620:  TCC CTG GAA ACC AAG AGT TTG GAA GTA ACC TTT ACT CCT GTC ATT GAG GAT ATT GGA AAA
       ser leu glu thr lys ser leu glu val thr phe thr pro val ile glu asp ile gly lys

 680:  GTT CTT GTT TGC CGA GCT AAA TTA CAC ATT GAT GAA ATG GAT TCT GTG CCC ACA GTA AGG
       val leu val cys arg ala lys leu his ile asp glu met asp ser val pro thr val arg

 740:  CAG GCT GTA AAA GAA TTG CAA GTC TAC ATA TCA CCC AAG AAT ACA GTT ATT TCT GTG AAT
       gln ala val lys glu leu gln val tyr ile ser pro lys asn thr val ile ser val asn

 800:  CCA TCC ACA AAG CTG CAA GAA GGT GGC TCT GTG ACC ATG ACC TGT TCC AGC GAG GGT CTA
       pro ser thr lys leu gln glu gly gly ser val thr met thr cys ser ser glu gly leu

 860:  CCA GCT CCA GAG ATT TTC TGG AGT AAG AAA TTA GAT AAT GGG AAT CTA CAG CAC CTT TCT
       pro ala pro glu ile phe trp ser lys lys leu asp asn gly asn leu gln his leu ser

 920:  GGA AAT GCA ACT CTC ACC TTA ATT GCT ATG AGG ATG GAA GAT TCT GGA ATT TAT GTG TGT
       gly asn ala thr leu thr leu ile ala met arg met glu asp ser gly ile tyr val cys

 980:  GAA GGA GTT AAT TTG ATT GGG AAA AAC AGA AAA GAG GTG GAA TTA ATT GTT CAA GCA TTC
       glu gly val asn leu ile gly lys asn arg lys glu val glu leu ile val gln ala phe

1040:  CCT AGA GAT CCA GAA ATC GAG ATG AGT GGT GGC CTC GTG AAT GGG AGC TCT GTC ACT GTA
       pro arg asp pro glu ile glu met ser gly gly leu val asn gly ser ser val thr val

1100:  AGC TGC AAG GTT CCT AGC GTG TAC CCC CTT GAC CGG CTG GAG ATT GAA TTA CTT AAG GGG
       ser cys lys val pro ser val tyr pro leu asp arg leu glu ile glu leu leu lys gly

1160:  GAG ACT ATT CTG GAG AAT ATA GAG TTT TTG GAG GAT ACG GAT ATG AAA TCT CTA GAG AAC
```

```
        glu thr ile leu glu asn ile glu phe leu glu asp thr asp met lys ser leu glu asn

1220:   AAA AGT TTG GAA ATG ACC TTC ATC CCT ACC ATT GAA GAT ACT GGA AAA GCT CTT GTT TGT
        lys ser leu glu met thr phe ile pro thr ile glu asp thr gly lys ala leu val cys

1280:   CAG GCT AAG TTA CAT ATT GAT GAC ATG GAA TTC GAA CCC AAA CAA AGG CAG AGT ACG CAA
        gln ala lys leu his ile asp asp met glu phe glu pro lys gln arg gln ser thr gln

1340:   ACA CTT TAT GTC AAT GTT GCC CCC AGA GAT ACA ACC GTC TTG GTC AGC CCT TCC TCC ATC
        thr leu tyr val asn val ala pro arg asp thr thr val leu val ser pro ser ser ile

1400:   CTG GAG GAA GGC AGT TCT GTG AAT ATG ACA TGC TTG AGC CAG GGC TTT CCT GCT CCG AAA
        leu glu glu gly ser ser val asn met thr cys leu ser gln gly phe pro ala pro lys

1460:   ATC CTG TGG AGC AGG CAG CTC CCT AAC GGG GAG CTA CAG CCT CTT TCT GAG AAT GCA ACT
        ile leu trp ser arg gln leu pro asn gly glu leu gln pro leu ser glu asn ala thr

1520:   CTC ACC TTA ATT TCT ACA AAA ATG GAA GAT TCT GGG GTT TAT TTA TGT GAA GGA ATT AAC
        leu thr leu ile ser thr lys met glu asp ser gly val tyr leu cys glu gly ile asn

1580:   CAG GCT GGA AGA AGC AGA AAG GAA GTG GAA TTA ATT ATC CAA GTT ACT CCA AAA GAC ATA
        gln ala gly arg ser arg lys glu val glu leu ile ile gln val thr pro lys asp ile

1640:   AAA CTT ACA GCT TTT CCT TCT GAG AGT GTC AAA GAA GGA GAC ACT GTC ATC ATC TCT TGT
        lys leu thr ala phe pro ser glu ser val lys glu gly asp thr val ile ile ser cys

1700:   ACA TGT GGA AAT GTT CCA GAA ACA TGG ATA ATC CTG AAG AAA AAA GCG GAG ACA GGA GAC
        thr cys gly asn val pro glu thr trp ile ile leu lys lys lys ala glu thr gly asp

1760:   ACA GTA CTA AAA TCT ATA GAT GGC GCC TAT ACC ATC CGA AAG GCC CAG TTG AAG GAT GCG
        thr val leu lys ser ile asp gly ala tyr thr ile arg lys ala gln leu lys asp ala

1820:   GGA GTA TAT GAA TGT GAA TCT AAA AAC AAA GTT GGC TCA CAA TTA AGA AGT TTA ACA CTT
        gly val tyr glu cys glu ser lys asn lys val gly ser gln leu arg ser leu thr leu

1880:   GAT GTT CAA GGA AGA GAA AAC AAC AAA GAC TAT TTT TCT CCT GAG CTT CTC GTG CTC TAT
        asp val gln gly arg glu asn asn lys asp tyr phe ser pro glu leu leu val leu tyr

1940:   TTT GCA TCC TCC TTA ATA ATA CCT GCC ATT GGA ATG ATA ATT TAC TTT GCA AGA AAA GCC
        phe ala ser ser leu ile ile pro ala ile gly met ile ile tyr phe ala arg lys ala

2000:   AAC ATG AAG GGG TCA TAT AGT CTT GTA GAA GCA CAG AAA TCA AAA GTG TAG CTAATGCTTG
        asn met lys gly ser tyr ser leu val glu ala gln lys ser lys val ***

2061: ATATGTTCAA CTGGAGACAC TATTTATCTG TGCAAATCCT TGATACTGCT CATCATTCCT TGAGAAAAAC AAT

2134: GAGCTGA GAGGCAGACT TCCCTGAATG TATTGAACTT GGAAAGAAAT GCCCATCTAT GTCCCTTGCT GTGAGC

2207: AAGA AGTCAAAGTA AAACTTGCTG CCTGAAGAAC AGTAACTGCC ATCAAGATGA GAGAACTGGA GGAGTTCCT

2280: T GATCTGTATA TACAATAACA TAATTTGTAC ATATGTAAAA TAAAATTATG CCATAGCAAG ATTGCTTAAAA

2352: TAGCAACAC TCTATATTTA GATTGTTAAA ATAACTAGTG TTGCTTGGAC TATTATAATT TAATGCATGT TAGG

2425: AAAATT TCACATTAAT ATTTGCTGAC AGCTGACCTT TGTCATCTTT CTTCTATTTT ATTCCCTTTC ACAAAAT

2498: TTT ATTCCTATAT AGTTTATTGA CAATAATTTC AGGTTTTGTA AAGATGCCGG GTTTTATATT TTTATAGACA

2571:  AATAATAAGC AAAGGGAGCA CTGGGTTGAC TTTCAGGTAC TAAATACCTC AACCTATGGT ATAATGGTTG AC

2643: TGGGTTTC TCTGTATAGT ACTGGCATGG TACGGAGATG TTTCACGAAG TTTGTTCATC AGACTCCTGT GCAAC

2716: TTTCC CAATGTGGCC TAAAAATGCA ACTTCTTTTT ATTTTCTTTT GTAAATGTTT AGGTTTTTTT GTATAGTA

2789: AA GTGATAATTT CTGGAATTAA AAA
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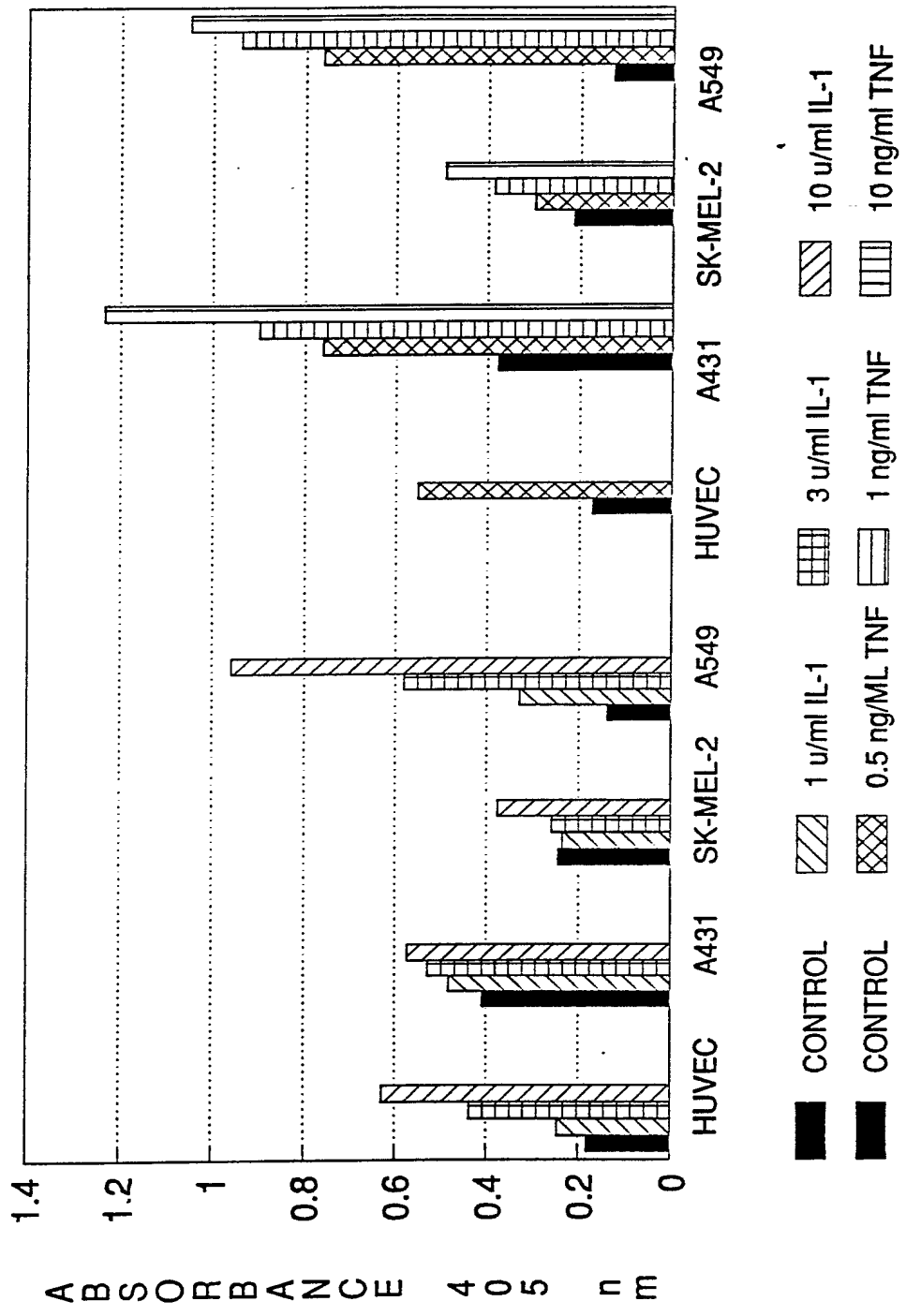

ICAM-1 INDUCTION
ABSORBANCE 405 nm
1.4
1.2
1
0.8
0.6
0.4
0.2
0
HUVEC
A431
SK-MEL-2
A549
HUVEC
A431
SK-MEL-2
A549
CONTROL
1 u/ml IL-1
3 u/ml IL-1
10 u/ml IL-1
CONTROL
0.5 ng/ML TNF
1 ng/ml TNF
10 ng/ml TNF

FIGURE 4

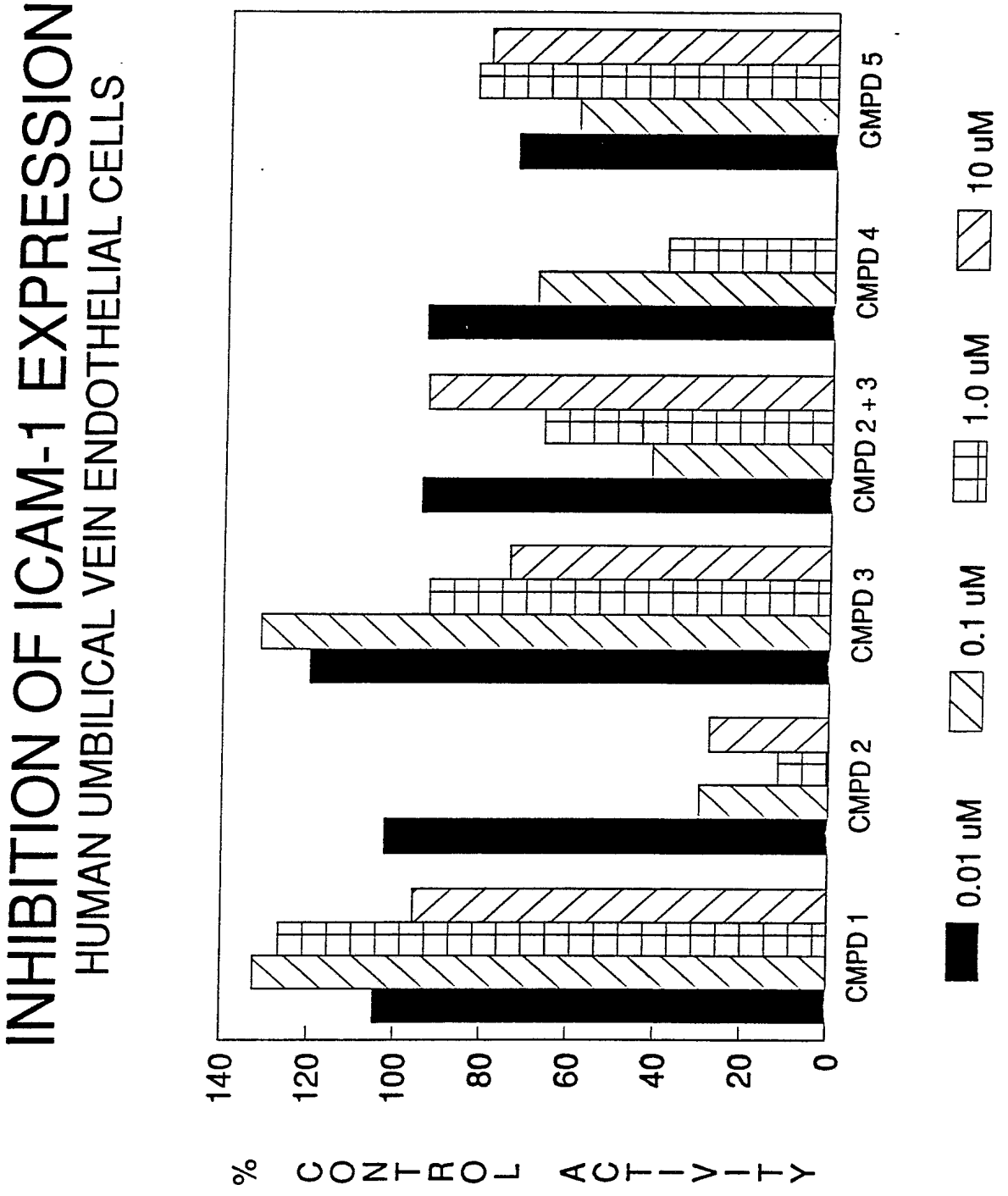

INHIBITION OF ICAM-1 EXPRESSION
HUMAN UMBILICAL VEIN ENDOTHELIAL CELLS
% CONTROL ACTIVITY
0
20
40
60
80
100
120
140
CMPD 1
CMPD 2
CMPD 3
CMPD 2 + 3
CMPD 4
CMPD 5
0.01 uM
0.1 uM
1.0 uM
10 uM

FIGURE 5

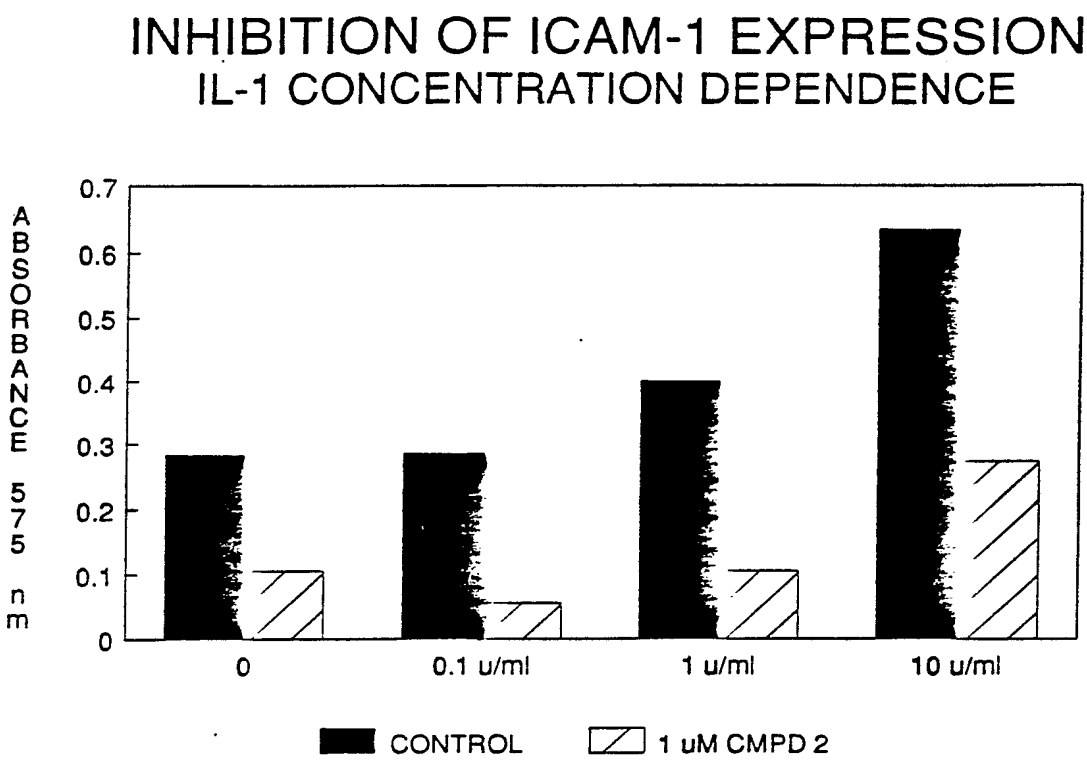


FIGURE 6A

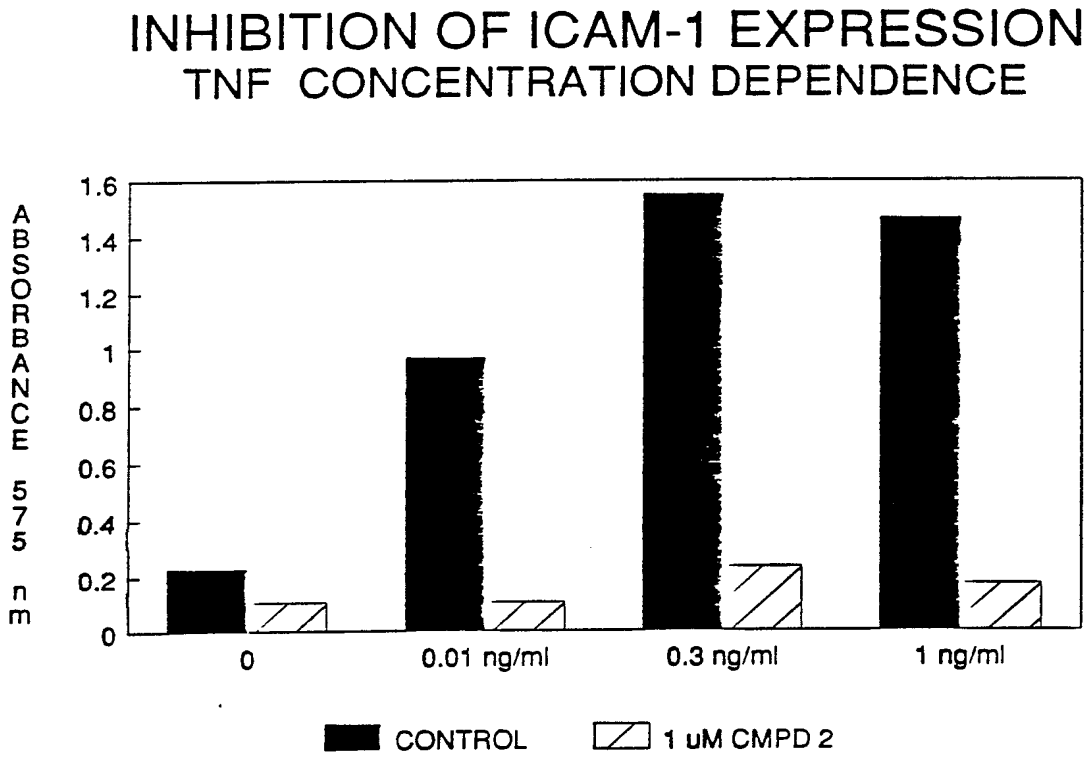


FIGURE 6B

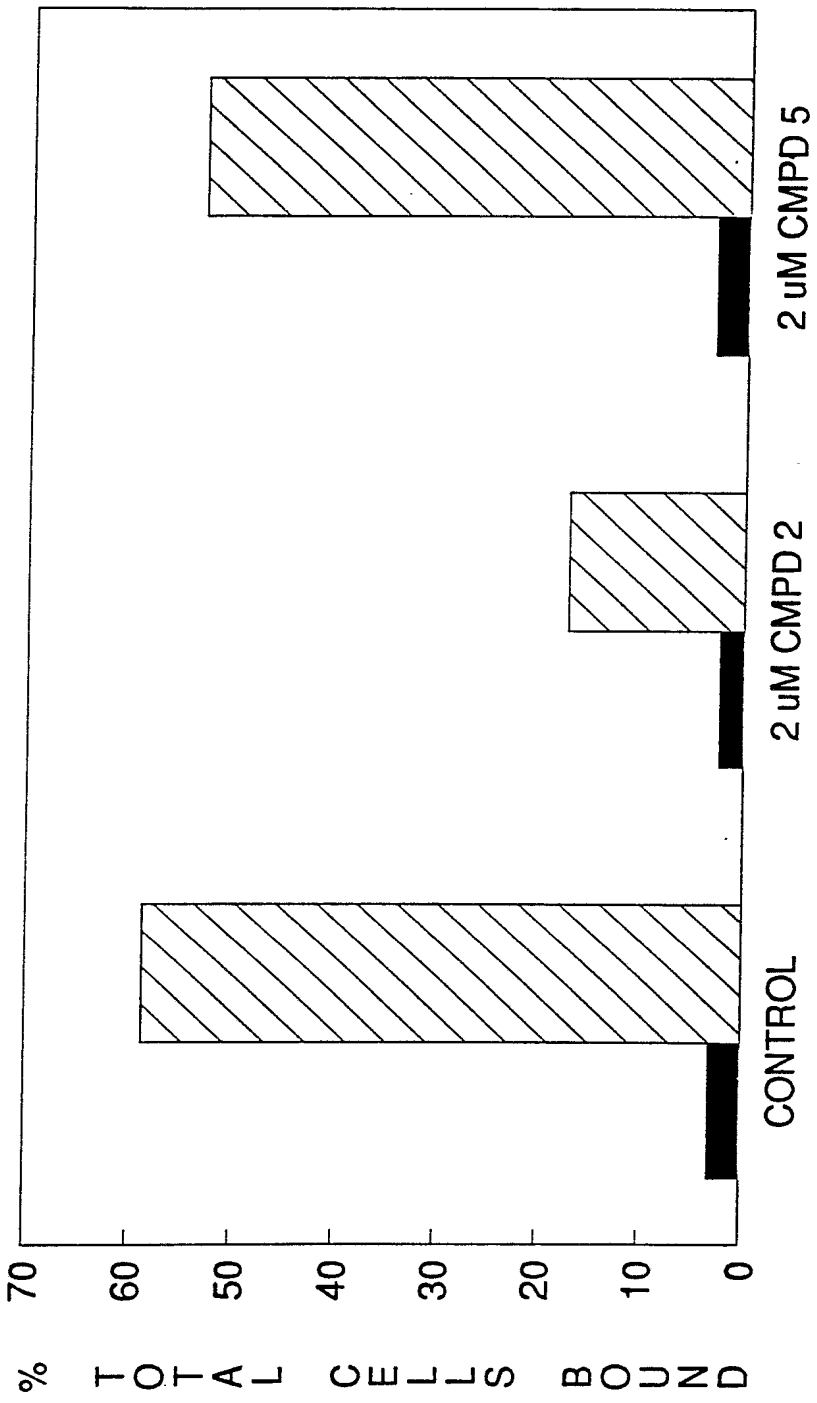

HL-60 ADHESION TO HUVEC
INHIBITION BY ANTISENSE OLIGONUCLEOTIDE
% TOTAL CELLS BOUND
0
10
20
30
40
50
60
70
CONTROL
2 uM CMPD 2
2 uM CMPD 5
BASAL
1 ng/ml TNF

FIGURE 7

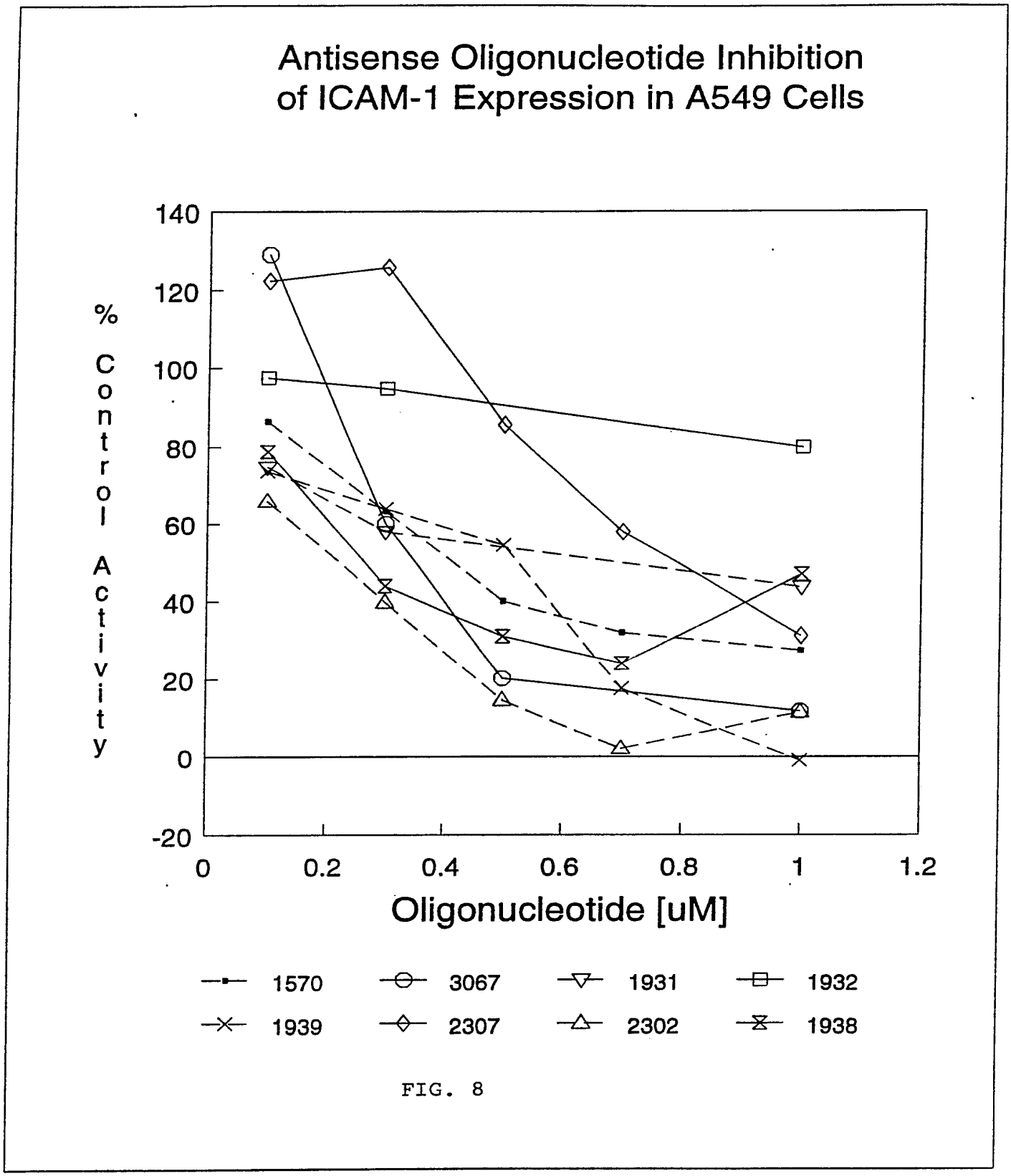

Antisense Oligonucleotide Inhibition
of ICAM-1 Expression in A549 Cells
% Control Activity
140
120
100
80
60
40
20
0
-20
0
0.2
0.4
0.6
0.8
1
1.2
Oligonucleotide [uM]
1570
3067
1931
1932
1939
2307
2302
1938

FIG. 8

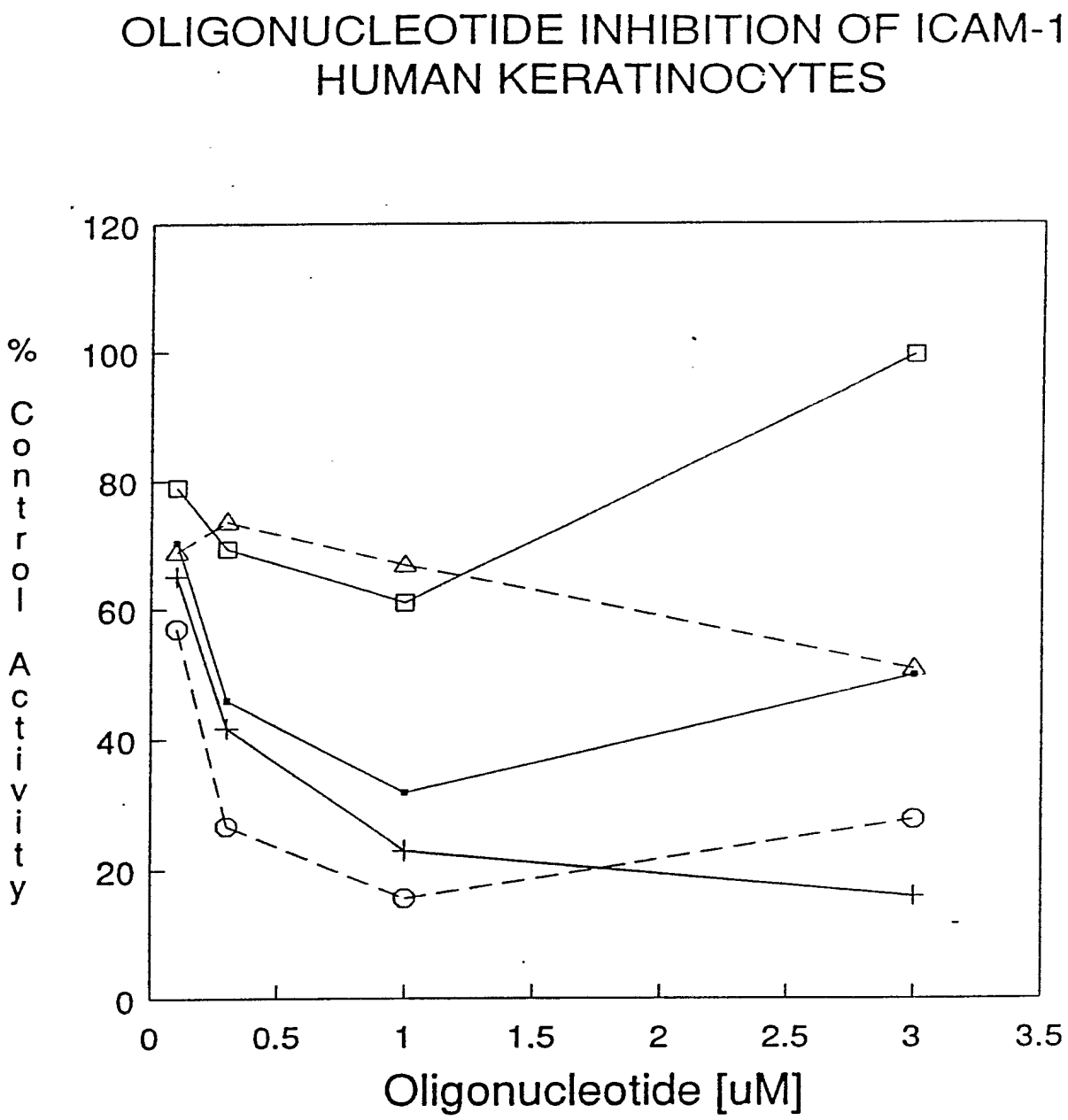

OLIGONUCLEOTIDE INHIBITION OF ICAM-1\
HUMAN KERATINOCYTES
% Control Activity
120
100
80
60
40
20
0
0
0.5
1
1.5
2
2.5
3
3.5
Oligonucleotide [uM]
1570
1939
1940
1821
2302
EXP 133

FIG. 9

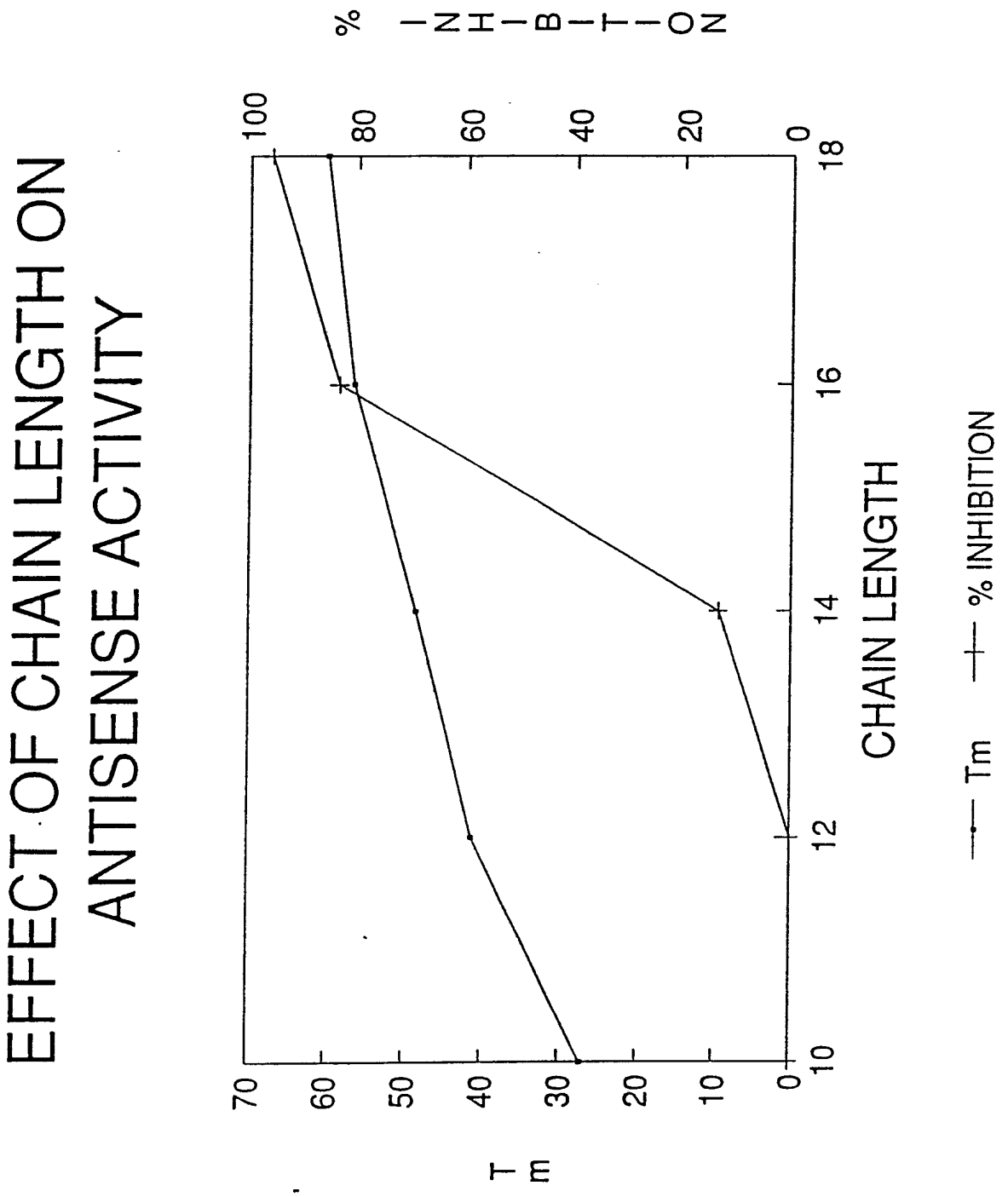
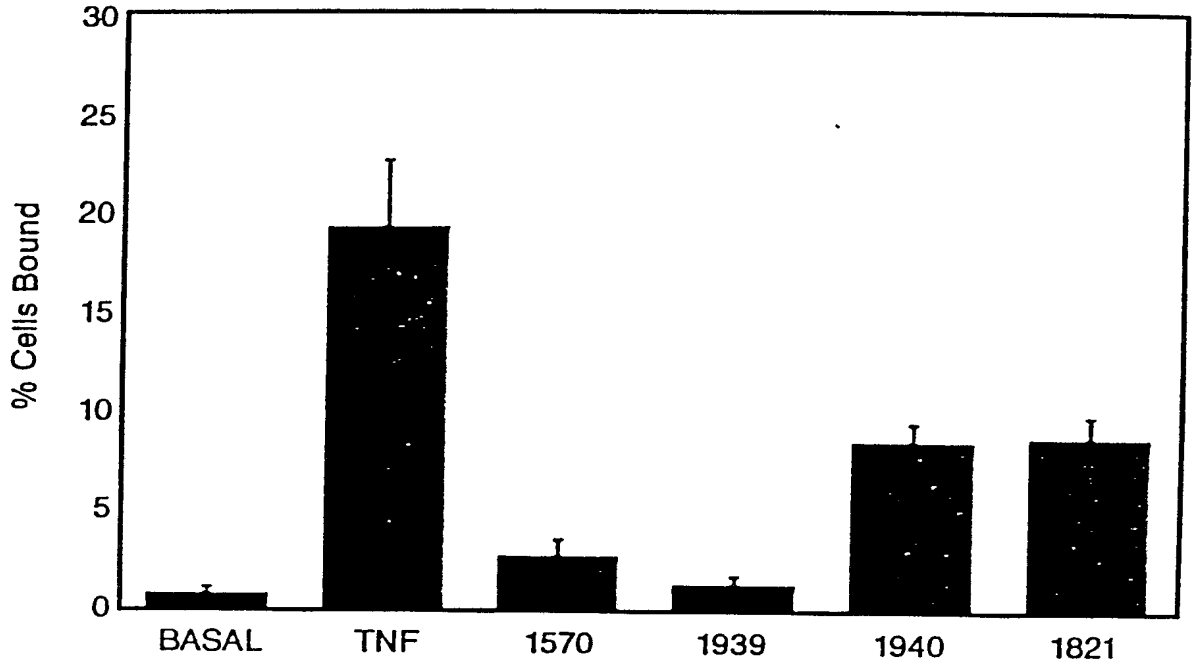

EFFECT OF CHAIN LENGTH ON ANTISENSE ACTIVITY
% INHIBITION
100
80
60
40
20
0
18
16
14
12
10
CHAIN LENGTH
Tm
70
60
50
40
30
20
10
0
Tm
% INHIBITION
100 nM OLIGONUCLEOTIDE

FIG. 10

FIG. 11



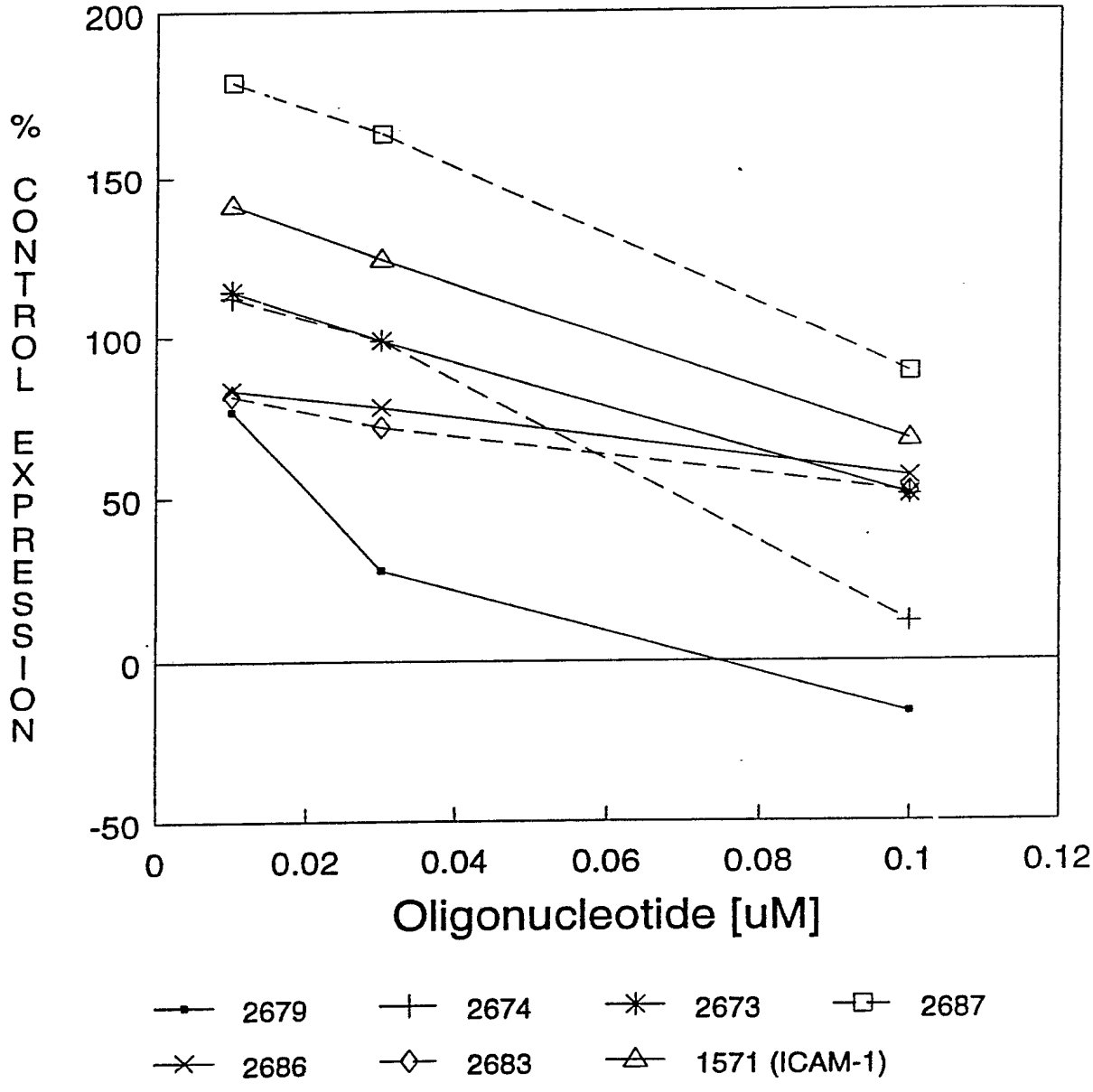

ANTISENSE OLIGONUCLEOTIDE INHIBITION
OF ELAM-1 IN TNF-a INDUCED HUVEC
% CONTROL EXPRESSION
200
150
100
50
0
-50
0
0.02
0.04
0.06
0.08
0.1
0.12
Oligonucleotide [uM]
2679
2674
2673
2687
2686
2683
1571 (ICAM-1)

FIG. 12

INTERNATIONAL SEARCH REPORT

International Application No. PCT/US91/05209

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) [6]

According to International Patent Classification (IPC) or to both National Classification and IPC

US: 514/44; 536/27

IPC(5): A61K 31/70; C07H 17/00

II FIELDS SEARCHED

Minimum Documentation Searched [7]

Classification System	Classification Symbols
US	514/44; 536/27

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched [8]

APS, GENBANK, UEMBL, CAS

III. DOCUMENTS CONSIDERED TO BE RELEVANT [9]

Category *	Citation of Document, [11] with indication, where appropriate, of the relevant passages [12]	Relevant to Claim No. [13]
Y	Pharmaceutical Research. Volume 5. No. 9 issued 1988. Zon. "Oligonucleotide Analogues as Potential Chemotherapeutic Agents". pages 539-549. see entire document.	1-5,9-12 26-29,33-35
Y	Cell. Volume 52. issued 25 March 1988. D.E. Stauton et al., "Primary Structure of ICAM-1 demonstrates Interaction between members of the Immunoglobulin and Integrin Supergene Families". pages 925-933. see entire documents.	6.22.30

* Special categories of cited documents: [10]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
07 OCTOBER 1991	28 OCT 1991
International Searching Authority	**Signature of Authorized Officer**
ISA/US	SUZANNE ZISKA

FURTHER INFORMATION CONTINUED FROM THE SECOND SHEET

V. ☐ OBSERVATIONS WHERE CERTAIN CLAIMS WERE FOUND UNSEARCHABLE [1]

This international search report has not been established in respect of certain claims under Article 17(2) (a) for the following reasons:

1. ☐ Claim numbers . because they relate to subject matter [12] not required to be searched by this Authority, namely:

2. ☐ Claim numbers , because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out [13], specifically:

3. ☐ Claim numbers_____, because they are dependent claims not drafted in accordance with the second and third sentences of PCT Rule 6.4(a).

VI. ☒ OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING [2]

This International Searching Authority found multiple inventions in this international application as follows:

Group I, claims 1-16 and 17-26
Group II claims 27-35

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims of the international application.

2. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims of the international application for which fees were paid, specifically claims:

3. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim numbers:

4. ☐ As all searchable claims could be searched without effort justifying an additional fee, the International Searching Authority did not invite payment of any additional fee.

Remark on Protest

☐ The additional search fees were accompanied by applicant's protest.

☒ No protest accompanied the payment of additional search fees.

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category *	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No
Y	Science, Volume 243, issued 03 March 1989, M.P. Bevilacqua et al., "Endothelial Leukocyte Adhesion Molecule 1: An inducible receptor for neutrophils related to Complement Regulatory Proteins and Lectins", pages 1160-1165, see entire document.	7.23.31
Y	Cell, Volumes 9, issued December 1989, L. Osborn et al., "Direct Expression coloning of Vascular cell adhesion Molecule a cyto kine-induced Endothelical Protein that binds to Lymphocytes" pages 1203-1211, see entire article.	8.24.32